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TITLE: EFFECT OF FOOD, DIET AND NUTRITION ON MILITARY READINESS AND PREPAREDNESS OF ARMY PERSONNEL AND DEPENDENTS IN A PEACETIME ENVIRONMENT

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Five projects underway at the Pennington Biomedical Research Center (PBRC) are reported herein. A clinical research laboratory is operational and supports U.S. Army Research Institute of Environmental Medicine (USARIEM) field research in sites ranging from Alaska to Bolivia. A stable isotope laboratory supports USARIEM research by determining energy expenditure in the field. The Fort Polk Heart Smart Project has completed an assessment of nutritional and exercise habits of military wives, a project that evaluates screening for cardiovascular risk factors and a project that assesses a health promotion model in military families. The Diet, Neurotransmitters and Behavior research team is conducting basic research in the effect of diet on behavior through beochemical, physiologic, and behavioral assessment studies. New studies assessing sleep deprivation and approaches to modifying ths stress through dietary manipulation are being initiated. The Menu Modification Project has analyzed and altered two sets of Army menus.

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Attachment 4

FOREWORD

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For the protection of human subjects, the investigator(s) have *adhered to policies of applicable Federal Law 45CFR46.*

In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

PI Signature: Donna N Lyon, MD Date: 8/12/91

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FINAL REPORT
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7/27/88 - 7/27/91

Background and Overview

On July 27, 1988, the U.S. Army awarded grant #DAMD-17-88-Z-8023, "The Effect of Food, Diet and Nutrition on Military Readiness and Preparedness of Military Personnel and Dependents in a Peace Time Environment" to the Pennington Center. The total grant award was $3,500,000. The U.S. Army staff at (USARIEM) reviewed the projects in August, 1988. The Committee on Military Nutrition Research reviewed and approved research projects proposed by the Pennington Centerat a site visit in 1988. The grant did not allow for equipment purchase and the Pennington Center used other funding sources to provide over $1.5 million equipment to support these research projects.

The results of this research have been completed on or ahead of schedule with high quality control and are detailed in annual and quarterly reports.

The Pennington Center developed four projects to meet U.S. Army objectives published in the Grant. They are detailed on the following page and listed below:

.Stable Isotope Laboratory for Determination of Energy Expenditure

.Support Laboratory for Human and Food Samples

.Menu Modification Project

.Fort Polk Heart Smart Project

In addition, Dr. Chandan Prasad developed a project with U.S. Army approval to study the effect of stressful situations and inadequate nutritional intake on performance. This project assembled a basic science team including senior investigator, neurophysiologist, neuroanatomist, behavioral assessment scientist, and neurobiochemist. This project is the **"Nutrition and Military Performance Laboratory."**

Two of the current research projects will terminate on schedule, on June 30, 1991. These two are the Fort Polk Heart Smart Project and the Menu Modification Project. As long as grant funds enable us, we will continue three of the projects initiated in conjunction with USARIEM. Those three projects are the Stable Isotope Laboratory, the Support Laboratory for Human and Food Samples Nutrition Services and the Nutrition and Military Performance Laboratory. We anticipate that all grant funds will be expended by December 31, 1991. If further funds are not provided

to support Army research at Pennington Biomedical Research Center, we must terminate those three projects on December 31, 1991.

Listed below are the Grant objectives and the projects developed by the Pennington Center that fulfilled those objectives.

US ARMY GRANT DAMD-17-88-Z-8023

Army Objectives	PBRC Projects to Meet Objectives
1. "Establish a Nutritional Health promotion Research Development Test and Evaluation (RDTE) Center for military personnel and dependents in a peacetime environment to accomplish the following: a. assess the nutritional adequacy of the diet of military personnel to promote health and military readiness; b. evaluate and develop military dietary programs for dining facilities, commissaries and other food service facilities operated by the military c. monitor the nutritional status of military personnel and their family members; and d. develop and evaluate military nutrition, education, and health promotion programs.	
2. Provide nutrition laboratory research support to the army's military nutrition research program at USARIEM to accomplish the following: a. provide biochemical assessment of nutrition status; b. perform food biochemistry analysis; and c. establish and perform stable isotope methodologies for assessment."	

We are indebted to the following army investigators for their assistance in this work: Colonel E. Wayne Askew, Colonel David S. Schnakenberg, Colonel Dale Block, Major Judy Turcotte, Colonel Garland McCarty, Colonel Fred Cecere, Ms. Doris Sherman, Colonel Denniston, Major Henley, Major Mays, Dr. Lieberman.

We were also assisted in the development of U.S. Army projects by former Acting Director of Pennington Biomedical Research Center, William Pryor, and Executive Director, George Bray.

Discussions of individual projects funded under this grant follow.

I. Clinical Research Laboratory

Introduction

One of the research objective of the Army grant is to "provide nutrition laboratory research support to the army's military nutrition research program at USARIEM to accomplish the following:

a. provide biochemical assessment of nutrition status;
b. perform food biochemistry analysis..."

The clinical research laboratory at Pennington Biomedical Research Center has dedicated itself to fulfilling these objectives. The laboratory has been equipped, employees hired, methods of analysis developed, and analyses performed for the research program at USARIEM.

Progress on Equipment

Equipment purchased and performing analyses during the period of this grant include the following:

a. Beckman Synchron CX5 automated clinical chemistry system;
b. Coulter STKS automated hematology system with five part differential;
c. Perkin Elmer P1000 ICP emission spectrometer;
d. Perkin Elmer Z5100 graphite furnace atomic absorption spectrophotometer with Zeeman background correction;
e. Antek Nitrogen analyzer;
f. Hewlett Packard 1090M HPLC system with diode array and fluorescent detectors, and autosampler;
g. Bio Rad HPLC system with electrochemical detector for catecholamine analysis;
h. Clinitek 2000 automated urine dip stick reader;
i. Packard RIASTAR 20 well gamma counter;
j. Hewlett Packard UV-Vis diode array spectrophotometer;
k. Hewlett Packard GC with IR detector;
l. Bruker ACE 250 NMR;
m. Bruker ESR system;

n. CEM microwave digestion system;
o. Hewlett Packard 1090IIM HPLC system with diode array and fluorescent detectors, autosampler, and method evaluation software (on bid).

General laboratory equipment and supplies have also been purchased and installed. These include centrifuges, air driven ultracentrifuge, refrigerators, ultralow freezer, incubating water bath, circulating cooling water bath, dry air incubator, analytical balances, pH meter, oven, microscopes, automatic pipets, freeze dryer, stirrer/hot plate, acid digestion system, lab rotator, and general laboratory supplies and glassware. In excess of $500,000 of Pennington center funds were used to equip this laboratory.

Progress on Method Development

a. General Chemistry

Most routine chemistry analyses are performed on the Beckman Synchron CX5 automated chemistry system. A description of this system is given in the Second Annual Report, pg 8 (1). Tests which we are currently performing on this system include:

glucose	albumin (BCP-humans)
urea	calcium
creatinine	phosphorus
sodium	magnesium
potassium	aspartate transaminase
chloride	alanine transaminase
carbon dioxide	alkaline phosphatase
uric acid	creatine kinase
total protein	lactate dehydrogenase
amylase	GGT
total bilirubin	direct bilirubin
cholesterol	HDL cholesterol
triglyceride	iron
iron binding capacity	albumin (BCG-animals)
free fatty acids	glycerol
beta-hydroxybutyrate	lactic acid
ammonia	alcohol
para amino benzoic acid	

Of the list above, tests which were developed at PBRC include free fatty acids, glycerol, beta hydroxybutyrate, lactic acid, ammonia, and para amino benzoic acid. Information on the details of methods for free fatty acids, glycerol, beta-hydroxybutyrate, lactic acid, and ammonia were given in the 1990 Annual report, Tables 1-3 (1). Recovery was nearly quantitative in all cases. These methods have been in use for a year and a half. Correlation studies for these are

presented in the appendix.

Four HDL cholesterol methods have been evaluated (heparin/Mn, isoelectric point phosphotungstic acid, Beckman phosphotungstic acid, and 50,000 molecular weight dextran sulfate. The heparin method was found to be unacceptable for use with the enzymatic cholesterol method on the CX5. The other three methods were correlated as shown in the appendix and Section I of the 1991 Annual Report (2). As suggested by Major Friedl we are switching over to the dextran sulfate method.

Para amino benzoic acid has been automated on the CX5 for the purpose of tracking food intake. Linearity, precision and recovery are good.

Vitamin A is being set-up on the CX5. This method is based on the reduction of iron/TPTZ [2,4,6-tris(2-pyridyl)-s-triazine] by ascorbic acid (3). The resulting product is measured at 593 nm (600 nm in our procedure). The method is made specific for ascorbic acid by blanking each sample after oxidizing the ascorbic acid with ascorbate oxidase. This blanking eliminates interference by other reducing materials in the sample. The method correlates very well with the dinitrophenyl hydrazine method. Our adaptation for the Beckman CX5 uses a total of 400 ul of sample, 200 ul for the total reducing materials and 200 ul for the blank. The two are run in separate tubes and the vitamin C concentration is obtained by subtracting the blank run from the total. The method is linear to 100 mg/L (see appendix). The major problem we have had to deal with is the fact that when reacting pure standards of ascorbic acid with ascorbate oxidase we obtain measurable ascorbic acid (6-8 mg/L for a 100 mg/L standard). This should be zero. It has been concluded that the problem is related to either:

1) incomplete conversion of ascorbic acid by the ascorbate oxidase, or

2) the presence of a non ascorbic acid component in the standard which also reduces the reagent.

Results which discourage the theory that the problem has to do with the enzyme include the following: the blank is not reduced by either 1) longer incubation times with the enzyme, or 2) using ten times the concentration of ascorbate oxidase.

We believe the problem may be related to the second hypothesis (non pure-standard), however, the manufacturer (Sigma) claims the ascorbic acid has at least 99% purity. Perhaps in agreement with this is the fact that we also used ascorbic acid from Bio Rad with the same results. We have

ordered new ascorbic acid and ascorbate oxidase from different companies. These will be tested when received. Work will continue on this method.

b. Nitrogen Analysis

Total urinary, fecal, sweat, water, or food nitrogen are measured on our model 703C pyrochemilluminescent nitrogen system (Antek Instruments, Inc., Houston, TX 77076). Urine samples are diluted approximately 1:121 prior to analysis. To analyze food or feces, a homogenate (0.2-1 g) is digested with 10 ml sulfuric acid, 1 ml hydrogen peroxide, and 1 ml of 10% cupric sulfate. The digest is diluted to 200 ml. Linearity , precision, and accuracy by recovery studies were determined for this method (Section I and the appendix of the 1991 Annual Report) (2).

c. Minerals and Trace Elements

Sweat, urine, water, food, and feces are analyzed on the Perkin Elmer P1000 ICP emission spectrometer. Sweat, urine, and water are analyzed directly or diluted with water. Feces and food are analyzed following homogenization and digestion with 10 ml of sulfuric acid, 1 ml of hydrogen peroxide, and 1 ml of 10% cupric sulfate. The digest is then diluted with water. It was found that when calibration was performed using aqueous standards in 0.1 mol/L nitric acid that there was a significant matrix effect due to the digestion solution. To counteract this standards were prepared in the digestion matrix. Results for linearity, precision, and recovery using these standards were given in Table 1 and the appendix of the 1991 Annual Report (2).

Trace element analysis is available for copper, chromium, manganese, zinc, selenium, aluminum, and platinum. A method for platinum has been developed for the graphite furnace atomic absorption spectrophotometer.

d. Coulter STKS

The Coulter STKS is now routinely used in the clinical laboratory. A study has been performed comparing the correlation of the five part differential with that obtained manually. These results are shown in the appendix. QC results compare very well to our peers and we have done very well in the CAP survey (appendix of the 1991 Annual Report) (2).

e. HPLC Methods

Work on amino acid separation by HPLC has been ongoing during the last year and a half of this grant (1). A very good separation was achieved. However, co-elution of GABA and arginine has caused problems. Several separations have been achieved, but failure to reproduce the separation between runs

has caused problems. Work will continue on this method.

A method for the analysis of caffeine was set-up by reverse phase HPLC. Verification of this method by assayed controls was performed.

We have begun developing a method of analysis for Vitamin A by reverse phase HPLC. The conditions thus far have consisted of using a mobile phase of 100% methanol through a C18 column. We have successfully separated retinol and retinyl acetate. Other metabolites have not been checked. When this occurs, we will fine-tune the procedure.

f. Radioimmunoassays

RIA's which were set up include ferritin, vitamin B12, red cell and serum folate, insulin, vasopressin, aldosterone, growth hormone, prolactin, and cortisol. These methods have all been evaluated by running assayed controls.

Progress on Quality Control

Procedure manuals for chemistry, urinalysis, quality control, and policies have been written and these policies and protocols put into practice. These are available for review at the Pennington Center. Quality control practices include routine monitoring of refrigerator and freezer temperatures, water quality, and reagent receipt and acceptability. Biannual checks of the linearity of each method, precision and accuracy of pipets, centrifuge speed and temperatures have been instituted (see appendix for examples of these protocols).

Routine quality control has been ongoing in the chemistry, hematology, immunoassay, and urinalysis sections. The chemistry and hematology internal QC results are compared monthly with other users of the same lot numbers across the country. We have generally rated very well on these reports. Examples of monthly reports for chemistry and hematology are included in the appendix of the 1991 Annual Report (2).

We have been subscribing the College of American Pathologist (CAP) external lab survey, as well as the Endo Survey of the American Association of Clinical Chemists since January, 1991. We have not reported results as yet for the Endo survey because we did not have the particular methods in service at the times of the survey. CAP survey results have been very favorable. Copies of these are also included in the appendix of the 1991 Annual Report (2).

Progress on Army Research Projects

The following studies have been completed for USARIEM:

a. Carbohydrate Load Study

A total of 51 samples were analyzed for ammonia, beta-hydroxybutyrate, glucose, glycerol, lactate, non esterified fatty acids, and triglyceride. In addition, 180 samples were analyzed for plasma lactate. In total, 557 tests were performed. The results for this study are shown in the 1990 Annual Report (1).

b. Alaska Winter Field Feeding Evaluation (1990)

A total of 156 samples were analyzed for Chemistry 22 panels plus HDL cholesterol. In total, 3588 tests were performed.

c. West Point Nutritional Assessment Study

Approximately 400 samples were obtained for the analysis of serum lipids, iron, TIBC, ferritin, Vitamin B_{12}, and folic acid. Also, 94 samples were analyzed for red blood cell folic acid. In all, 1645 analyses were performed. The report is shown in the appendix of the 1990 Annual Report (1).

d. Sodium Depletion Study

The sodium depletion study was completed this year. Nitrogen, sodium, potassium, calcium, magnesium, and phosphorus were measured in urine, sweat, water, feces, and food. Due to the discovery of a matrix interference, the analyses were repeated a second time for the minerals on the food and feces. These were done using standards containing the digestion matrix (sulfuric acid/hydrogen peroxide/cupric acetate). Captain Moore indicated to us that the results for the MRE samples was too low for sodium. He asked that we investigate the problem. Half of the original MRE samples were repeated using a different digestion mixture (nitric acid/hydrogen peroxide). In the meantime, it was discovered that a mathematical error had been made in some of the food samples. Some foods had been homogenized with an equal weight of water; this water had not be taken into account in the final calculations. Doing so doubled all of the mineral weights per gram of food and total concentration of each mineral. All of the MRE samples and some of the other foods were processed this way. An amended report was prepared. The second set of digested MRE's matched the corrected concentrations of mineral from the first digestion very well. In addition, a second set of MRE foods were received from Natick in order for us to check our methods. We digested these in the same manner as the first set (sulfuric acid/hydrogen peroxide/cupric sulfate). The results for MRE foods which were the same as the first shipment agreed very favorably with the first batch (2). A total of 637 samples

(urine, water, sweat, feces, and food) were analyzed for sodium, potassium, calcium, magnesium, phosphorus, and nitrogen for a grand total of 3822 tests.

e. Alaska 91

Serum samples from the Alaska 91 study were received and 61 samples were processed for glucose, BUN, creatinine, total protein, cholesterol, triglyceride, HDL, lactate, non esterified fatty acids, beta hydroxybutyrate, and glycerol in serum and creatinine and nitrogen in urine. The report is shown in the appendix of the 1991 Annual Report (2). A total of 61 samples were analyzed for a grand total of 500 tests.

f. Pikes Peak

Urine samples from the Pikes Peak study were received this year and processed for urinary nitrogen and creatinine. The report is shown in the appendix of the 1991 Annual Report (2). A total of 177 tests were performed on 97 samples.

g. Bolivia High Altitude Study

A total of 116 urine samples were analyzed for nitrogen and creatinine in this study (232 tests).

h. Survival Study

Serum samples from the Survival Study were received and analyzed for a chemistry panel, glycerol, lactic acid, and HDL cholesterol. All analyses have been completed, however the report has not yet been compiled. Most samples were repeated at least once due to the very abnormal results found. These results were confirmed in most cases. 60 samples were analyzed for a total of 1500 tests.

i. Ranger Study

The first shipment from the Ranger study was received in July. These samples have been frozen and are awaiting analyses. Included will be a chemistry panel and HDL cholesterol, beta hydroxybutyrate, glycerol, lactate, free fatty acids, iron, TIBC, ferritin, serum folate, RBC folate, vitamins A, C, and D, red cell AST, transketolase, and glutathione reductase. Almost 1700 samples were received for this study (including duplicate or triplicate samples). Three more shipments are expected within the next few months. It is planned to have all results finished by November of this year.

On the following page Table 1 tabulates the studies and number of tests performed in each study.

References

1. Effect of Food, Diet, and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependent's in a Peacetime Environment, Second Annual Report (8/1/89-7/31/90), August 15, 1990.

2. Effect of Food, Diet, and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependent's in a Peacetime Environment, Third Annual Report (7/29/90-7/28/90), 1991.

3. Liu TZ, Chin N, Kiser MD, Bigler WN, Specific spectrophotometry of ascorbic acid in serum or plasma by use of ascorbate oxidase. Clin Chem 28 (11), 2225-2228 (1982).

TABLE 1. Studies Performed and/or Received by the Clinical Research Laboratory at PBRC.

Study	No. Samples	No. Tests	When Completed
Carbohydrate Load	231	551	January 1990
Alaska 1990	156	3588	March 1990
West Point	494	1645	July 1990
Bolivia High Alt.	116	232	October 1990
Alaska 1991	61	500	April 1991
Pikes Peak	97	177	May 1991
Sodium Depletion	637	3900	Aug 1991
Survival Study	60	1500	Aug 1991
Ranger Study	~1000?	~3700?	
TOTAL STUDIES	~2852	~15,793	

II. Stable Isotope Laboratory

Introduction

Establishment of a Stable Isotope Laboratory to support the Army's military nutrition research program at USARIEM was a research objective of US Army grant DAMD 17-88-G-8023. The Stable Isotope Laboratory at Pennington Biomedical Research Center was established in September, 1989 with the employment of James P. DeLany, Ph.D., as manager of the laboratory. A Finnigan Delta S Isotope Ratio Mass Spectrometer, a water-CO_2 equilibrator, a Breath Carousel for CO_2 Analysis, a Gas Chromatograph/Combustion Interface and a Multiport automatic tube cracker were purchased using USDA

funds. The instrument was installed and calibrated, and the first Army samples analyzed by April, 1990. A Research Associate, Stable Isotope position was filled by Teodora Aranas, who began May 14, 1990.

The research conducted by the Stable Isotope Laboratory has been in the area of energy requirements of soldiers under harsh environmental conditions. The conditions studied have been in an arctic climate (2 studies) and at altitude. The method used to determine energy requirements was to determine expenditure using the doubly labeled water technique.

The use of doubly labeled water for measurement of energy expenditure was developed as a field technique for use in small animals (1). The method is based on the premise that after a loading dose of $^{2}H_{2}{}^{18}O$, ^{18}O is eliminated as CO_2 and water, while deuterium is eliminated from the body as water. The rate of CO_2 production, and, hence, energy expenditure, can be calculated from the difference of the two elimination rates. Doubly labeled water, using the two-point method, is an ideal method for use in free-living subjects because it is noninvasive and nonrestrictive. The only requirement of subjects is to give urine and saliva specimens before and after drinking an initial dose of $^{2}H_{2}{}^{18}O$, and then return in one to two weeks to give a final urine specimen. An interim specimen is often collected in addition to initial and final specimens. During the period between the two urine and saliva samplings, subjects are free to carry out their normal activities and are not required to maintain extensive diaries. Although these characteristics have been taken advantage of by zoologists for 20 years, doubly labeled water has only recently been applied for determination of energy expenditure in free-living human subjects (2-4)

The doubly labeled water method has been extensively validated in humans under controlled settings (5), but there are confounding factors that need to be considered in field studies, particularly in Army Field Studies. Among these are change in location or food and water supply immediately preceding, or during an energy expenditure study. These changes may cause a change in baseline isotope abundance and, therefore, interfere with the accuracy of the energy expenditure measurement. This has occurred in a previous field training exercise involving the study of the MRE and RLW rations (2). Therefore, a group not receiving labeled water must be followed to make any corrections in baseline isotope shifts.

Doubly Labeled Water Method

Total body water is calculated using ^{18}O isotopic enrichments measured predose, and 3 and 4 hours after the dose as follows:

$$TBW = (A/MW_d)(APE_d/100)18.02[1/R_{std}(E_s-E_p)](1/1.01)$$

where A is the dose given in grams, MW_d is the molecular weight of the dose water, APE_d is the atom percent excess enrichment of the dose water, R_{std} is the ratio of heavy to light isotope of SMOW, i.e., 2.005×10^{-3}, E_s and E_p are the enrichments of the final and predose samples. The final step in the equation, division by 1.01, is necessary since the ^{18}O dilution space is larger than TBW (6).

The mean daily CO_2 production (rCO_2, mole/day) was calculated according to Schoeller et al. (5):

$$rCO_2 = (N/2.078)\ (1.01k_O - 1.04k_H) - 0.0246rH_2O_f$$

where N is the average of the beginning and end of period total body water and rH_2O_f is the rate of water loss via fractionating gaseous routes, and is estimated to be $1.05N(1.01k_O - 1.04k_H)$. The ^2H and ^{18}O isotope elimination rates (k_H and k_O) were calculated using the initial and final time points (two-point method). In the Alaska90 Cold Weather Study and Bolivia90 high altitude study, linear regression using the isotopic enrichment relative to predose of the first two days and last three days of the metabolic study were also used to determine elimination rates. There has been some controversy regarding the accuracy and precision of the two-point and regression methods. The advantage of the two-point method is that we obtain the true elimination rate even during changing physiologic conditions (which often occurs in Army Field Studies). The advantage of the multipoint regression methods is improved precision from averaging out analytical error.

Energy expenditure is calculated by multiplying rCO_2 by the energy equivalent of CO_2 calculated from the macronutrient content of each diet, and body stores of protein and fat used for energy (7).

Isotopic analyses. The ^{18}O isotope abundances were measured on a Finnigan Delta S gas-inlet Isotope Ratio Mass Spectrometer with a CO_2-Water equilibration device. Briefly, urine and saliva samples were equilibrated with CO_2 at 18 °C in a shaking water bath for at least 10 h. The CO_2 is then cryogenically purified under vacuum before introduction into the mass spectrometer. The hydrogen isotope abundances were measured on a Finnigan Delta S gas-inlet Isotope Ratio Mass Spectrometer, as previously described (2). Briefly, urine and saliva samples were distilled under vacuum into tubes containing zinc reagent (Friends of Biogeochemistry, Bloomington, Indiana). The reduction tube were sealed with a flame and placed in a 500 °C oven for 30 minutes to reduce the water to hydrogen gas which is then introduced into the mass spectrometer.

Progress and Major Scientific Achievements

The major scientific achievements for the Stable Isotope Laboratory have been the measurement of energy in two Arctic Field

Training Exercises and one high altitude training exercise using the doubly labeled water procedure. A summary of the three projects is given in the table below. Detailed descriptions of the studies are presented in the following sections.

Study	Total Daily Energy Expenditure (kcal/day)
Alaska90	5170 ± 630
Bolivia90	3550 ± 610
Alaska91	4250 ± 480

Alaska90 Cold Weather Study

The deuterium and ^{18}O enrichment of 6 urine samples between February 4, and February 14 were analyzed in the six unlabeled subjects. There were no significant shifts in baseline isotope abundance in the unlabeled group. The deuterium and ^{18}O enrichment of 6 urine samples and 6 saliva samples were analyzed for the 14 labeled subjects. The elimination rates were calculated by the two point method, using the initial and final enrichments, as well as a regression method (5 time points). The analyses for one subject (#104) were repeated to determine the analytical precision. For ^{18}O the coefficient of variation for the elimination rate by the 2-pt method was 0.4% while for regression it was 0.8%. The CV for the dilution space was 0.4% and 0.1% for the initial and final time points. The elimination rates calculated by the 2 point method and the regression method were similar in some instances but considerably different in others. For deuterium, the coefficient of variation for the elimination rate by the 2-pt method was 1.2% while for regression it was 1.6% for the repeat analysis. As for ^{18}O the elimination rates calculated by the 2 point method and the regression method were similar in some instances but considerably different in others.

An average RQ and energy equivalent of CO_2 were estimated by calculating an FQ from the protein, carbohydrate and fat intake of each soldier (See 9th Quarterly Report). Energy expenditure was then calculated from the deuterium elimination rates and the ^{18}O elimination rates and dilution spaces which were determined last quarter. The average daily energy expenditure of the 14 labeled soldiers was 5143±595 kcal by the 2-point method and 4847±498 by the regression method. The reason for the difference between the two methods is that the regression method is sensitive to systematic changes in energy expenditure. In this study the soldiers were stood down on one day and underwent strenuous physical activity the next, and both of these time points were used in the regression analyses. When using the regression method, this causes the elimination rate obtained from the slope of the line to be skewed as well as the 0-time intercept used to calculate dilution spaces. These errors then cause an error in energy expenditure. The two-point method, however, is not affected and provides the true elimination rates. The dilution spaces are

obtained by the plateau enrichment 4-hours after the dose.

Lean body mass (LBM), calculated by isotope dilution (TBW/.73) decreased from 64.9±5.3 at the beginning of the period to 64.3±5.2 by the end of the metabolic period. Body fat calculated from the difference between body weight and LBM only decreased by 1.8 kg during the study. This decrease is lower than the true fat loss, due to the timing of the final weighing. Initial weights were taken in the morning between 0600-0800 hours, while the final body weights were taken in the afternoon between 1500-1700 hours. Therefore, the final body weights would be higher than they would have if taken first thing in the morning. Since fat weight is obtained by subtracting LBM from total weight, if the total weight appears higher than it truly is, then the fat weight will be overestimated. This fact is clearly demonstrated by the considerably lower energy expenditure calculated by the Intake/Balance (I/B) method. For this method the energy intake plus changes in energy content of the body (fat and protein) are combined to obtain energy expenditure. The average energy intake was 3059±784 kcal, the change in fat and protein energy content were 684±2781 and 82±327 kcal, giving an energy expenditure of only 4316±1087 kcal/d, which is over 800 kcal/d lower than that obtained by doubly labeled water.

Bolivia High Altitude Study

The deuterium and ^{18}O enrichments of urine samples (6 and 5 time points respectively) of 6 subjects in the placebo group of the Bolivia high altitude study have were analyzed. Unlike the Alaska90 study, there was a significant decrease in enrichment over time. There was a shift in ^{18}O of -2.33 o/oo and in deuterium of -23.8 o/oo. The average decrease was used to correct the enrichment of the labeled subjects when calculating elimination rates. This correction is needed to obtain the correct energy expenditure during changing isotopic enrichment of the water supply. The deuterium elimination rate (Kd) is the average of two separate analyses for each subject. The deuterium analyses were repeated for two reasons: 1) because this was the first full project for the technician, and 2) to determine the accuracy of the determination of Kd, because deuterium is the most difficult analytical aspect of the method.

The calculations using the 2pt and regression method yielded almost identical means and standard deviations, 3549±608 vs 3565±674. The energy expenditures calculated from the repeat deuterium analyses also yielded nearly identical energy expenditures using either the 2pt or regression methods, with a coefficient of variation of about 2% (See 11th Quarterly Report). There were some problems with samples from subject #59, both in energy expenditure and total body water, and hence, was excluded from the mean calculations. The energy expenditure results have been published in USARIEM Technical Report No. T10-91 (8).

Alaska91 Cold Weather Study

The deuterium and ^{18}O enrichments of urine samples of 5 subjects in the placebo group were analyzed. As in the Alaska90 study, there was no significant change in enrichment over time. Since the data from the Bolivia study demonstrated that the energy expenditure obtained using the 2pt and regression methods yield the same results, only the 2pt method was used in this study. The mean energy expenditure of the 10 labeled soldiers was 4250 ± 480 kcal/d. There was a problem with the first measurement of total body water for 4 of the subjects, it appears that there were problems with the measurement of the ^{18}O dose the soldiers received and in 2 cases problems with collection of saliva samples. Therefore, the RQ was assumed to be 0.823 and the estimate for the energy equivalent of CO_2 was 5.8 kcal/L.

Ongoing Projects

The Stable Isotope Lab is presently involved in 2 Army research projects. One is a water turnover study, part of the Fairchild Air Force Base Survival Study. For this study, urine and saliva samples are being analyzed for deuterium to determine total body water at the beginning and end of the study, and for water turnover throughout the study. Analyses of these samples has begun, and should be finished shortly.

The other project currently underway is the Rangers Training Study, in which energy expenditure will be measured using doubly labeled water. There are four parts of this study, the Fort Benning phase (7/26/91 - 8/10/91), the Mountain phase (8/11/91 - 8/28/91), the Swamp phase in Florida (8/29/91 - 9/13/91), and a final Desert phase (9/14/91 - 9/26/91). The samples for this study will be analyzed as each phase of the study is completed.

Conclusions

The doubly labeled water method has proven to be an ideal method for the measurement of energy expenditure of soldiers during field training exercises. The two-point method, in which elimination rates are measured from isotope enrichments of urine samples from the first and last days of the study has proven to be valid in these studies. The only requirements of the soldiers is give urine and saliva samples and drink the heavy water. The energy expenditure of soldiers during the Arctic Field Training exercises was higher than anticipated, particularly in the Alaska90 study. The energy expenditures during the Alaska90 study were considerably higher than during the Alaska91 study (5170±630 vs 4250±480 kcal/d). This is not surprising in light of the facts that it was considerably colder during the Alaska90 study, the soldiers were more active and needed to wear snowshoes more during the Alaska90 study, and the soldiers did not move their artillery as much as had been anticipated during the Alaska91 study. The energy expenditure

at altitude during the Bolivia study was essentially the same as was anticipated.

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III. Diet, Neurotransmitters, and Behavior

Introduction

The current scientific staff of the Neuroscience Laboratory includes Chandan Prasad, Ph.D., Jeffery W. Brock, Ph.D., Shakeel Farooqui, Ph.D., Anwar Hamdi, M.D., Ph.d., and Masahiro Sakata, M.D. They are devoted full-time to research on the Army Grant DAMD 17-88-Z-8023. There are presently four students in the laboratory, working 20 hour/week schedules.

Major Scientific Achievements, 1988-1991

* First development of an antibody to dopamine D_2 receptor protein.

* First evidence that the dopamine D_2 receptor is coupled to a subunit of GTP-binding protein.

* First evidence that transcriptional regulation has only a minor, or secondary, role in the expression of dopamine D_2 receptors in the brain.

* First quantitative evidence that long-term, high dietary-protein results in an increase in motor activity and increased basal arousal levels.

* First definitive evidence that dietary protein influences the cellular morphology and functional state of the cerebral cortex.

* First to devise the methodology for separating and measuring all known serotinin metabolites in a single analysis.

* First definitive evidence that circulating cyclo(His-Pro), a neuropeptide that is known to modulate many types of behavior, is not derived from dietary protein.

Background.

The focus of the neuroscience laboratory program is to apply the expertise of the current research staff to investigate the role of nutrition in behavior. Projects have been undertaken which include behavioral, neurophysiological, and molecular neurobiological measurements to study the effects of macronutrient manipulations on higher brain function. Overall, the research has broad application to problems related to aging and development, mental function and dysfunction, as well as to the questions of nutrition science. In the interest of taking a state-of-the-art, multidisciplinary approach to solving scientific problems, Dr. Prasad has assembled a team of scientists drawn from different specialities and possessing expertise in a variety of scientific methods. The scientific staff has included: Shakeel M. Farooqui, Ph.D. in Biochemistry, with expertise in Molecular Biology; Jeffery

W. Brock, Ph.D. in Physiology, with expertise in Neurophysiology; Emmanuel S. Onaivi, Ph.D. in Pharmacology, with expertise in Behavioral Pharmacology; Anwar Hamdi, M.D., and Ph.D. in Pharmacology, with expertise in Behavioral Pharmacology; and Masahiro Sakata, M.D., with expertise in Molecular Biology. Students who have worked with the Neuroscience Lab at different times are: C. Z. Chuang, Lisa Theriot, Joseph LaFleur, Ashley Cowart, Stephanie Tarlton, Sheila Venugopal, Shorye Payne, Keith Ross, and Lori Adams.

The Neuroscience Laboratory research program has focused on basic and applied research, utilizing a number of techniques in molecular biology, neurochemistry, pharmacology, and neurophysiology. A summary of the basic and applied research, and the application are presented:

1) Applied Research

* Diet, brain chemistry, and behavior
* Nutritional factors in centrally acting pharmaceutical agents
* Higher brain function

2) Basic Research

* Regulation of dopaminergic neurons
* Neurochemistry
* Molecular biology
* Neuronal function

The studies which have been performed over the past two years are topically related to problems of:

* Mental performance, function, and dysfunction,
* Aging and development,
* Neurological and mental disorders eg., Parkinsonism and schizophrenia

The Neuroscience Lab has enhanced its methodological capabilities by joining in collaborative research with the Gene Expression Laboratory and the Analytical Laboratory at the Pennington Biomedical Research Center. The rewards have not only been an advancement of our own research efforts, but a demonstration of our value as a resource for others working in the area of nutritional neuroscience. The Neuroscience Laboratory currently is developing in a new research direction, investigating the effects of stress on cognitive function and the potential for nutritional intervention to protect against stress-related cognitive deficits.

General Progress

Project 1: Behavioral Neurochemistry of Food-derived Peptides (Chandan Prasad). We have chosen three peptides to be included under this program: i) cyclo(His-Pro), CHP, ii) casein-derived peptides (exorphins), and iii) delta-sleep inducing peptide, DSIP (a peptide known to reduce blood pressure and protect against stress response). The first phase of this study has largely concentrated on i) The relationship between diet and endogenous cyclo (His-Pro) levels, and ii) the mechanism of action of cyclc (His-Pro) in the striatum, an area of the brain actively involved in motor coordination.

CHP has been shown to exist in a variety of tissues and biological fluids such as the brain, GI tract, blood, CSF, and semen, etc. While CHP-like immunoreactivity from such biological specimens has been characterized chromatographically, in no case has the peptide been isolated in enough quantity and purity that its presence can be ascertained by physical methods. We, for the first time, have isolated pure CHP from human urine and determined its structure to be histidyl-proline diketopiperazine. These data have been accepted for publication in "Biochemistry International".

Having established the existence of CHP in a biological fluid, we have focused our attention on the question, "Could dietary proteins serve as cyclo (His-Pro) precursors?". To this end, we have examined the urinary levels of CHP in three species- a carnivore (leopard), an herbivore (rhinoceros), and an omnivore (man). The data from these studies suggest that urinary levels of CHP is higher in animals consuming high levels of dietary proteins. However, in these studies data on exact composition of diet at dietary levels CHP was not available. Therefore, we subjected rats to three different diets (of known chemical composition with undetectable level of CHP): carbohydrate-rich, caesin-rich, and whey-rich.

Proteins in casein and whey have 13 and 3 Pro-His or His-Pro sequences. If both of these proteins were to be hydrolyzed in such a way as to release all His-Pro of Pro-His sequences, animals on caesin-diet should excrete at least 4 times more CHP than those on whey diet. This hypothesis is also consistent with the observation that exogenous CHP rapidly clears from the plasma and accumulates in the urine. However, the results from this study show that the differences in the plasma or urine levels of CHP in rats on these three different diets (carbohydrate, caesin, and whey) were insignificant. Our conclusion is that endogenous CHP may not be derived from the metabolism of ingested dietary proteins.

In a related study, we have examined the presence of CHP in 12 common nutritional supplements using partial protein hydrolysates. Nine out of 12 samples contained CHP. Those supplements with the highest CHP levels had undergone more intense thermal manipulation

prior to packaging than others. Furthermore, oral administraiton of one of these supplements (Ensure) to a human volunteer resulted into a rapid rise in plasma levels of CHP. In conclusion, these data show that while it is unlikely that CHP may be derived from dietary proteins, a diet containing hydrolysed protein (or CHP) may contribute to endogenous levels of CHP.

Project 2: Cyclo(His-Pro) and food intake (Anwar Hamdi and Jeffery Brock). Administration of exogenous CHP to rats and mice has been shown to elicit many endocrine and central nervous system-related biological activities. CHP in a dosage of 2.5 uM/rat/day is known to produce a 20% ($p<0.05$) reduction in daily food intake. Consistent with the appetite-modulating effects of the peptide is the observation that fasting elevates the hypothalamic CHP content which then returns to normal after feeding. Until recently, the inhibitory effects of CHP on food intake had been demonstrated using a mixed diet only. It is well known, however, that rodents can regulate their macronutrient intake when presented separately with carbohydrate (C), protein (P), and fat (F) diets. This led us to investigate whether intraventricularly administered CHP may affect caloric intake and, if so, would the changes in caloric intake be due to alterations in the intake of all or only some of the macronutrients.

To accomplish this, rats were allowed to choose from C-, P-, and F-rich diets to display macronutrient preferences after vehicle or CHP infusion into the cerebral ventricles. Ninety percent of the calories in C-, P-, and F-rich diets were derived from C, P, and F, respectively, with the remaining ten percent of the calories derived from equal parts of the other two macronutrients. Fisher 344 rats (400-450 gm) were implanted with indwelling intraventricular canulae, housed individually, and then allowed to recover from surgery for 4-5 days. Rats were fasted from food, but not water, for 21 hours and then allowed to consume three macronutrients presented separately for a total period of 3 hours. Total energy intake (Kcal/Kg/3 hrs) and percent of total energy derived from each macronutrient was calculated on five consecutive days prior to vehicle or peptide (CHP, 1-methyl CHP, or 3-methyl CHP) administration.

Administration of saline (vehicle) alone led to an appreciable increase in total caloric intake, which was characterized by increased preference for fat and decreased preference for both carbohydrate and protein. These changes after vehicle administration may be due to non-specific stress resulting from handling and intraventricular perfusion. On intraventricular administration of CHP (0.5 uM/Kg), but not 1-methyl CHP or 3-methyl CHP, both total caloric intake ($p=0.0075$) and fat preference ($p=0.0354$) decreased, whereas carbohydrate preference increased ($p=0.0518$), with no change in protein preference ($p=0.2458$). In conclusion, these data show that CHP differentially modulates macronutrient selection. Therefore, endogenous CHP in the central

nervous system may play a role in regulation of food preferences.

Project 3: Determination of tryptophan metabolites using HPLC (Chandan Prasad and C. Z. Chuang). The amino acid tryptophan is an initial substrate for brain serotonin synthesis which is not easily transported across the blood brain barrier. Serotonin activity has potential consequences throughout the CNS. Thus, the study of the pathways for tryptophan metabolism is critical to an understanding of cerebral serotonin function in a number of behavioral systems. There are at least two major pathways for tryptophan metabolism. The first pathway leads to the decarboxylation of tryptophan. The second pathway, the pyrotase pathway, results in the formation of metabolites following cleavage of the indole ring. At least two of the metabolites of the tryptophan pyrotase pathway - quinolinic acid and kynurenic acid - have been reported to play important roles in excitatory neurotranmission, neurotoxicity, and epilepsy. Kynurenine has also been shown to act as an excitotoxin in in vitro studies.

Studies into the role of other tryptophan metabolites in the pathogenesis of human neurological disorders are limited by the availability of technology to separate and quantitate these compounds in biological samples. Quinolinic acid, a tryptophan metabolite with a major role in CNS function, has previously not been included with other metabolities in analytical procedures; therefore there is a need for a new sensitive method that can separate and measure many tryptophan metabolities, including quinolinic acid, in a single sample. In order to optimize the conditions for the simultaneous separation of the tryptophan metabolites, a reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed. This involved the sequential optimization of the mobile phase, by adjusting the pH, the concentration of of triethylamine and the gradient elution. The baseline resolution of the compounds by this optimized procedure was obtained with an analysis of time, including the re-equilibration period of less than 30 minutes. This research, which has been accepted for publication in the Journal of Chromatography, is the first RP-HPLC method that can separate tryptophan and its metabolites in a single chromatographic run. A copy is enclosed as an appendix.

Project 4: Preparation and characterization of dopamine D_2 receptor protein antibody (Shakeel Farooqui and Jeffery Brock). Dopamine plays an important key role in brain function. Abnormalities in the metabolism of dopamine in specific regions of the brain are associated with mental and neurological disorders, such as schizophrenia and Parkinson's disease. In order to study these molecular disturbances we raised antibodies in rabbits against the dopamine receptor type D_2. Two peptides corresponding to amino acid sequence predicted from the nucleotide sequence of the dopamine D_2 receptor were chemicaly synthesized. These are Peptide 1 (CGSEGKADRPHYC) and Peptide 2 (NNTDQNECIIY) corresponding to 24-

36 and 86-98 from the NH2 terminal. The peptides were conjugated with a keyhole limpet hemocyanin using glutraldehyde and the conjugate was injected to rabbits. The polyclonal antiserum was obtained and screened for specific antipeptide 1 or antipeptide 2 antibodies on ELISA. Antibodies against peptide 1 showed high titer for peptide 1 with little or no cross reactivity with the other peptides. The antibodies were further characterized on a Western blot. Peptide 1 antibodies reacted with denatured D_2 receptors from rat striatal membranes, Mr 91 kDa. The preimmune sera or peptide 2 antibodies did not show any band corresponding to 91 kDa. Peptide 1 antibodies were further characterized for immunoinhibition studies using D_2 specific ligands. Peptide 1 antibodies significantly (40%) inhibits the photoaffinity labeling of D_2 receptor by 125I-NAPS. Such an interaction of antibody with native D_2 receptor was further studied using a D_2 specific ligand (3H) YM-09151-2. (3H) YM-09151-2 binding was significantly inhibited (35-40%) by the addition of peptide 1 antibodies. The addition of preimmune or pooled rabbit serum did not show an inhibition in the YM binding. These results suggest that anti D_2 receptor antibodies binds to dopamine receptor either on the ligand binding site or in close proximity, which results in the inhibition of ligand-receptor interaction.

Project 5: Determination of dopamine (D2) receptor messenger RNA expression (Masahiro Sakata and Shakeel Farooqui). The application of this technology has a major contribution in the study of central dopaminergic mechanisms which are implicated in a number of neurological and mental disorders. Our laboratory has made rapid progress in establishing the protocol for the determination of the dopamine D2 receptor mRNA expression using Northern blot analysis. Using the modified guanidium thiocyanate method, the total mRNA extracted was denatured at 55 C in 50% formamide, (6% formaldehyde solution for 15 minutes and electrophoresed in 1% agarose / 0.66M formaldehyde gels. After electrophoresis, RNA on the gels was transferred to nylon filters and the filter was baked at 80°C for 2 hours. The filter was prehybridized for one hour at 42°C in 50% formamide, 0.25M NaCl, 0.25M sodium phosphate (pH 7.2), 1mM EDTA and 0.1% SDS in a volume of 10ml and hybridized for 24 hours at 42°C with 1.0x106 c.p.m./ml 32-P-labeled PD2 cDNA in the same hybridization buffer. The membranes were washed at room temperature in a solution containing 2xSsc and 0.1% SDS (200ml) and then twice at 65°C in 200ml 0.1ml 0.1xSSC and 0.1% SDS. Autoradiography was done at -70°C for one day. All northern blots were probed also for B-actin mRNA content, as control.

The D2 mRNA that was detected using the PD2-DNA probe was 2.6kb and was prominent in pituitary and striatal tissues, but was not seen in the testes and liver. As positive control using the B-actin probe, strong bands were detected following hybridization in all samples indicating a successful RNA preparation. With this capability, a number of problems can now be investigated in the laboratory, such as: 1) the expression of D2 mRNA following dietary

and other physiological and pharmacological manipulations relevant to the aging process, 2) the mechanism of regulation of the D2 mRNA expression in animal models and its control, and 3) dopamine agonists and antagonistic effects and the effects of dietary manipulation on the D2 mRNA.

Project 6: Dopamine D2 receptor protein antibody mapping in the rat brain (Jeffery Brock). This study was undertaken to demonstrate the utility of the antibody as a D2-selective label by determining the distribution of antibody binding in situ. Rat brains were dissected and sliced on a freezing microtome into 40 micron sections. Every fourth section was incubated with D2-selective antisera, following the procedure of peroxidase-anti-peroxidase (PAP) immunocytochemical labeling. Alternate sections were incubated with pre-immune sera and antisera plus free oligopeptide. The anatomical distribution of PAP complex was verified in selected sections which were stained with cresyl violet, and compared to Paxinos' "The Rat Brain in Stereotaxic Coordinates".

PAP complex staining was heavy in the striatum, frontal and parietal cortices. PAP complex was distributed throughout the basal forebrain and stained the heaviest in the olfactory tubercle, medial septal nuclei and nuclei of the diagonal band In the hypothalamus, lighter, diffuse staining continued throughout, with heaviest precipitate in the periventricular area. Staining was evident throughout the medial forebrain bundle all the way back to the ventral tegmental area and the substantia nigra pars compacta. Heavy staining was observed in the dorsal thalamic nuclei and in the medial habenula, and more caudally in the midbrain, the central gray area around the Aquaduct of Sylvius. Unexpectedly, PAP was seen in lateral thalamic nuclei, also in the superior and inferior colliculi. This previously undescribed distribution of D2 receptors may belong to the periaquaductal dopaminergic system, which have their cell bodies of origin in the zona compacta and send afferents to the dorsal midbrain.

Sections incubated with primary antiserum followed by free peptide antigen in concentrations greater than 10 microgm/ml showed no precipitate in any of the above mentioned areas. Inhibition of specific antibody binding in this way resulted in little or no contrast staining, and an appearance identical to those sections incubated with pre-immune serum. These data are consistent with the distribution of efferent dopaminergic fibers, and the binding distributions of known D2 receptor radioligands. Also, these data are in good agreement with the areas known to express D2 mRNA, which has been found in the highest concentrations in the neostriatum, olfactory tubercle, substantia nigra, ventral tegmental area, nucleus accumbens, and the intermediate lobe of the pituitary gland. These data suggest that D2 receptor protein was recognizable at all levels of the dopaminergic system, i.e., target tissue, axons, and cell bodies of origin.

Project 7: Dietary protein and behavior in rats (Emmanuel Onaivi and Jeffery Brock). Many studies indicate that dietary macronutrients are important determinants of brain function. Both undernutrition and overnutrition in preweaning rats result in persistent functional alterations of the brain. The effects of protein undernutrition on behavior, brain development, and intellectual function are well known. Unfortunately, reports on the effects of high dietary protein on animal behavior are largely anecdotal. For example, it has been observed that adult rats consuming a high-protein diet become more easily frightened and "snappish". In addition, much of our knowledge about the effects of high dietary protein on behavior is inferred from studying the effects of tyrosine administration, based upon the understanding that brain tyrosine is increased by a single meal in proportion to its protein content. The issue is complicated by the observation that brain levels of tyrosine are not increased with long-term consumption of high dietary protein, apparently due to an adaptational decrease in total intake by the animal. Thus, tyrosine administration may not accurately model the effects of long-term, high-dietary protein on behavior.

In order to better understand the role of dietary protein in behavior, more precise and quantitative assessments of behavior must be made in animals consuming different levels of protein. Investigators currently have a battery of behavioral tests at their disposal which are both quantitative in nature and, in some cases, characterized with regard to known neurotransmitter(s) involved in mediating the behavior. The adhesive patch test and negative geotaxis are commonly used to evaluate sensorimotor function in rats; locomotor activity and stereotypy are inversely related measurements of ambulatory, or searching, behavior in rats; the tail-flick test is widely used to measure nociception in rats and mice; activity in the elevated plus-maze is a well characterized index of aversion behavior and anxiety. In the present study, these tests were used to evaluate the effects of varying levels of dietary protein on behavior in adult rats.

In the first phase of these experiments, groups of rats were pair-fed with isocaloric diets containing normal-(20%), low-(8%) and high-(50%) casein for 20 weeks. In assessing the performance of rats in the battery of behavioral test systems the animals on the high-protein diet were more responsive in sensorimotor function, negative geotaxis and spontaneous locomotor activities when compared to normal and the low protein groups. These rats showed a reduced aversion in the elevated plus-maze test which has been extensively used to study anxiolytics and anxiogenic drugs. In the tail-flick reaction time to a heat stimulus, analgesia was produced in animals fed the low-protein diet while hyperalgesia was induced in animals on the high-protein diet.

Project 8: Levels of dietary protein and modification of behavioral responses to CNS acting drugs (Emmanuel Onaivi). An elaborate study was undertaken to determine the effects of long-term dietary protein manipulation on the behavioral effects of some centrally acting drugs. In this study, mice were used and placed on one of the three isocaloric diets for 35 weeks: high-protein (HP), normal-protein (NP), low-protein (LP). The diets consisted of 50, 20, and 8% casein, respectively. The rest of the calories in the diet were made up with cornstarch and sucrose, and equal amounts of fat. All three diets were supplemented with salt and vitamin mixture and choline bitartrate. During the treatment period, the animals' body weights were not significantly different in the three groups. Locomotor activity and stereotypy following the administration of the vehichle or amphetamine (0.1 and 1.0 mg/ml), which were measured using the opto-varimex mini system (Columbus Instruments, Ohio). The data was analyzed using one-way ANOVA followed by Dunnett's q' test.

Both spontaneous locomotion and stereotypy increased as the level of protein in the diet increased ($p<0.05$, N=6 per group). The NP fed animals exhibited a slight decrease in locomotion at low amphetamine but significantly increased at the higher dose of amphetamine. In contrast, LP animals showed significant increase in locomotion at both amphetamine doses. The stereotypic response after amphetamine in the LP or NP animals exhibited similar pattern as the locomotor activity. These results suggest that central dopamine receptors were altered by the long-term dietary protein manipulation and consequently modified the amphetamine induced behavior.

The next series of experiments were designed to further assess the influence of the long-term dietary protein manipulation and the consequences on the behavioral performance following the administration of a neuroleptic, fluphenazine, and the psychoactive constituent of marijuana, delta-9-THC. In this experiment, male ICR mice weighing 20-25 grams were housed in a temperature controlled room with reversed 12:12 hr light/dark cycle. The animals in the following groups were fed equicaloric diets, A: low-protein (8% casein); B: high-protein (50% casein); and C: medium protein (20% casein) for 35 weeks.

Animals in the different groups were injected intraperitoneally (ip) with the vehicle, delta-9-THC, or fluphenazine. The performance of the animals in a number of behavioral test systems was evaluated following the administration of the vehicle of drug regimen: fluphenazine (0.01-0.5 mg/kg), delta-9-THC (1-30 mg/kg). The vehicle or drug were administered for 40 minutes prior to behavioral analysis. The spontaneous locomotor activity of mice was monitored in individual activity cages following vehicle or drug treatment. The computer-controlled system was designed to monitor the total as well as ambulatory counts. The stereotype response was deduced from the difference between the total and

ambulatory counts.

The dietary protein manipulation modulated mouse motor behavior with the spontaneous locomotor activity of the animals on the high protein diet increased by about 50% ($p<0.05$). In naive animals, delta-9-THC or fluphenazine produced a dose dependent inhibition of mouse spontaneous locomotor activity. The high-protein diet increased the mouse sensitivity to the locomotor inhibitory effects of fluphenazine or delta-9-THC.

The Pertwee ring test was utilized to assess catalepsy and data expressed as an immobility index. All animal were assessed for a total of five minutes and the time each animal remained motionless on the ring was recorded. In naive mice, fluphenazine or delta-nine THC induced a dose dependent state of immobility. It was observed that the dietary protein manipulation modified the catalepsy induced by fluphenazine or delta-9-THC. The high protein diet influenced the cataleptogenic sensitivity to fluphenazine or delta-9-THC, as compared to the low or medium protein fed animals.

The tailflick reaction time to a heat stimulus was determined after vehicle or delta-9-THC administration. A ten second maximum latency was set to prevent tissue damage. The change in latency for each animal was computed and expressed as % MPE (% possible effect), where % MPE was determined using the following method: {(test latency-control latency)/ (10 seconds-control latency)} x 100. The long-term dietary manipulation increased the mouse sensitivity to the effects of delta-9-THC.

The computer controlled two compartment black and white box, as well as the elevated plus maze was used to determine the stress/anxiety index following different diets. The exploratory activity in the black and white chambers as well as the number of transitions were recorded in a 5 minute test session. The feeding of the high- and low-protein diets reduced and increased, respectively, mouse aversion in this test which is known to be sensitive to anti-anxiety drugs. The dopamine antagonist, fluphenazine, induced catalepsy, inhibited stereotypy, and reduced mouse spontaneous locomotor activities. A similar pattern was recorded with delta-9-THC, demonstrating modification of murine behavior following manipulation of dietary protein.

The results taken together suggest that the CNS function which can be influenced by long term dietary protein alteration, may modify those receptors that are sensitive to the effects of delta-9-THC. Furthermore, the central dopamine receptor function was altered by the dietary protein manipulation. It is unlikely that the mechanism of action underlying the behavioral modification induced by delta-9-THC or fluphenazine following the dietary manipulation are the same.

Project 9: Dietary protein and dopamine receptor regulation (Anwar Hamdi and Emmanuel Onaivi). We have collected a large body of evidence that dietary-protein manipulations have definite effects on higher brain function in animals, with evidence accumulating from behavioral, neurophysiological, and neurochemical studies. Preliminary data from our lab suggests that these effects include changes in neurotransmitter receptor populations in the central nervous system. Binding data was collected using the rat striata of the 8% casein, 20% casein, and 50% casein diet groups. The data indicated a 30% decrease in D2 receptor binding in the group of animals that were fed the low protein diet. The low-protein diet group also demonstrated a reduction in striatal dopamine receptor protein compared to controls, as indicated by D2-selective antibodies recently developed by our lab. Animals which were fed the high-protein diet demonstrated more dense antibody binding on immunoblot, suggesting that more dopamine receptor protein was present in the sample. Strangely, in the binding studies, Bmax for the high-protein group was not different from control. In total, there is evidence that central dopaminergic mechanisms may be respectively facilitated or reduced by an increase or decrease in dietary-protein levels.

Project 10: Effects of dietary protein on monoamines and monoamine metabolites (Shakeel Farooqui, Jeffery Brock, Emmanuel Onaivi). The digestion products of food serve not only as the building blocks of brain structures and as energy sources, but also as the starting materials for neurotransmitter synthesis. Proteins are particularly important foodstuffs for the brain, since their breakdown products, the amino acids, serve as precursors of almost all known neurotransmitters. There is ample evidence that peptides derived from dietary proteins play a role in modulating behavior. However, studies by most previous investigators in the area of nutrition and behavior have been scanty and inconclusive. Therefore, the determination of monoamines, dopamine, norepinephrine, serotonin, and their metabolites following dietary protein manipulation has been undertaken.

In the first series of experiments, 5 groups of animals were placed on one of five diets: 8% casein ad lib, 20% casein ad lib, 50% casein ad lib, 20% casein pair-fed, and 50% casein pair-fed. After the animals had been on their respective diets for 8 months, all were sacrificed by rapid decapitation and their brains were stored at -80°C. Brains selected from the 8%, 20% pair-fed, and 50% pair-fed animals were sliced using a freezing microtome. Thirty-six areas of the brain were collected by punch-dissection and processed for analysis by high-performance liquid chromatography/electrochemical detection. Analyses of 4 major neurotransmitters (dopamine, serotonin, norepinephrine, epinephrine) and 3 metabolites (HVA, 5-HIAA, DOPAC) in each of the tissue-punch samples are presently underway.

Preliminary data suggests that there are distinct abnormalities in dopamine levels in the brain. There are increases in dopamine levels in some dopaminergic areas, while other dopaminergic areas show a decrease. Although the precise pattern of dopamine changes are not clear at present, it will be interesting to determine if different dopaminergic central neural circuits are affected selectively by the dietary manipulation.

Project 11: Dietary protein and preparatory arousal in rats (Jeffery Brock). Previous investigators have observed that rats fed high-protein diets (50-80% casein) are easily frightened and demonstrate more violent behavior than rats on control diets. Data from our laboratory have shown that rats fed a chronic, high-protein diet (50% casein) are more reactive to nociceptive stimuli than those fed either normal-or low-protein diets (20 and 8% casein, respectively). The mechanisms underlying these changes are unknown. One theory is that high-dietary protein increases tyrosine availability for the synthesis of central catecholamines which, in turn, increase arousal levels in the animal. A weakness of this theory is that tyrosine levels are not elevated in the rat brain at dietary-protein levels of up to 80% casein.

The Cortical Negativity Response is an electrical correlate of the Alerting Reaction and preparatory arousal levels. This negative shift in cortical slow potentials is easily recordable even in anesthetized animals, when the animal is conditioned using an alerting stimulus-imperative stimulus paradigm. Cortical Negativity Responses were successfully recorded in urethane/chloralose anesthetized rats which were fed either a 20% or 50% casein diet for 36-40 weeks. There were two identifiable negative deflections, designated N1 and N2, which occurred after the alerting stimulus and before the imperative stimulus. Each peak was analyzed with regard to latency, amplitude, and duration. N1, which is generated by the frontal cortex as an orienting response to alerting stimuli, was not different between the two diet groups. However, the N2 deflection, which is generated by the motor cortex, was significantly prolonged in latency and higher in amplitude in the high-protein diet group. It is known from primate studies that the amplitude of this deflection is related to the subject's basal arousal level and the subject's preparation, or intention, to move. Furthermore, N2 amplitude is directly correlated with dopaminergic activation in the central nervous system. Additional testing revealed no differences between groups with regard to somatosensory evoked potentials and short-latency brainstem auditory evoked responses. These results suggest that the high-protein diet caused an increase in preparatory arousal mechanisms, which was not accompanied by changes in sensory information processing. These data are consistent with the theory that high-protein diets cause an over expression of catechol-aminergic mediated behavior.

Project 12: Dietary protein and neuronal plasticity (Jeffery Brock). In the relatively short time that the Neuroscience Laboratory has been operational, we have collected a large body of evidence that dietary-protein manipulations have effects on higher brain function in animals. There is reason to believe that central dopaminergic mechanisms are facilitated or inhibited, respectively, by an increase or decrease in dietary-protein levels. The available evidence has accumulated from behavioral, neurophysiological, pharmacological, and neurochemical studies. Such a multi- disciplinary approach is necessary, given the complexity of the brain; no one method can provide sufficient information to describe adequately the changes that occur after nutritional manipulations.
Combining descriptive morphology with neurotransmitter level measurements, etc., may provide further insight into the complex processes underlying behavior.

Changes in neurotransmitter and postsynaptic membrane protein synthesis alter the functional properties of synapses. These may be accompanied by alterations in synaptic structural properties as well. Although functional deficits of the brain can be caused by changes which are not reflected in gross morphology, recent works have shown that synaptic densities in certain areas of the brain are modified by nutrition.

Under certain conditions, synapses show plasticity, i.e., a change in their pattern of structural or functional connectivity. In the cerebral cortex, 80-90% of axons terminate on dendritic spines. In general, dendritic spines constitute as much as 96% of the surface area of pyramidal cells in the cerebral cortex and it is now well-accepted that dendritic spines play an important role in mechanisms of behavior, including learning, general states of alertness, and mentation. The purpose of the present study is to determine if the dramatic functional and biochemical changes which result from manipulating dietary protein are accompanied by changes in dendritic spine density. This is important because a decrease in dendritic spine density, i.e., a deficit in synaptic connections, would provide a simple causal explanation for the relationship between nutrition and functional impairment.

The Rapid Golgi method, which involves incubating brain tissue with osmium dichromate and silver nitrate, offers the opportunity to view single neurons with all their processes stained. This stain penetrates to very few cells in the tissue, but makes visible the details of axonal and dendritic ramifications not seen with usual histological techniques. This allows one to compare fine details of dendritic morphology between normal and experimental animals.

The present study was undertaken to determine if the dramatic functional and biochemical changes which result from manipulating dietary protein are accompanied by changes in dendritic spine

density. Brains were dissected from different groups of rats which consumed 8%, 20%, or 50% casein diets for 4 weeks. The tissues were fixed in 10% formalin and sliced into 150 micron sections, using a freezing microtome. The sections were stained using the Rapid Golgi method and dendrites were visualized by Nomarski differential phase-contrast microscopy. Dendritic spine densities were determined for the following areas: frontal cortex, parietal cortex, entorhinal cortex, striatum, and septum. Spine densities were statistically analyzed using single factor Analysis of Variance, followed by Student's t-tests. Statistical significance was accepted at the 95% confidence level (alpha = 0.05, two-tailed test).

In animals maintained on 50% casein for 4 weeks, dendritic spine densities were significantly increased in all 5 brain areas investigated ($p<0.05$), compared to the control group (animals maintained on 20% casein). For the animals maintained on the 8% casein diet for 4 weeks, dendritic spine densities were only significantly different in the striatum and entorhinal cortex, being elevated in both areas compared to the control animals. In the animals consuming the 8% casein diet, spine densities in the frontal cortex, parietal cortex, septum were not different from control animals. These rather surprising results suggest that dendritic spine density is sensitive to levels of protein in the diet; however, the relationship between dietary protein and brain cell morphology is not a simple covariance. This non-linear effect on spine density induced by the dietary manipulation suggests that protein undernutrition and overnutrition stimulate different physiological mechanisms in the brain. The possibility is underscored by the observation that, not only did protein undernutrition and overnutrition have non-linear effects on specific brain areas, but the responses were also different between brain areas. These differences probably reflect the neural and biochemical individuality of each area.

Our understanding of the physiological roles of these specific brain areas in their contribution to behavioral expression provide for some interesting speculation as to the mechanisms involved in the effects of dietary protein on behavior. In the case of the 8% casein-fed animals, increased spine density in the striatum and entorhinal cortex may reflect an increase in food-searching behavior in the animal while sparing general cognitive function. In the case of the 50% casein-fed animals, the over-development of dendritic spines in the cerebral cortex, striatum, and septum suggests a widespread increase in neuronal excitability in the brain. An increase in neuronal excitability in these key brain areas may be the neural substrate of the behavioral hyperactivity and hyper-responsiveness previously observed in animals maintained on long-term, high-protein diets.

Project 13: Dietary protein and microtubule-associated proteins (Shakeel Farooqui and Jeffery Brock). This investigation is intimately related to the subject of neuronal plasticity, mentioned above. Dendritic spines are dynamic structures that are capable of very rapid structural modification. These shape changes involve actin which is present in the spine in a microspecialized configuration that permits local contraction or extension of the cell membrane. Actin networks are isotropic gels and actin gel-solution transitions are under the control of local calcium concentrations. Others have shown that actin gel-solution transition may also involve the differential phosphorylation of microtubule-associated protein (MAP) subtypes, MAP1, MAP2, and MAP-tau. MAP2 is expressed exclusively in the brain and is highly localized in neuronal soma and dendrites, whereas MAP1 and tau are expressed throughout the cell. The phosphorylation of high molecular weight MAP2 appears to be a dendrite-specific event that is required for neuronal plasticity; it is believed that interaction between MAP2 and actin allows for rapid cytoskeletal rearrangements within dendrites. In contrast, MAP1 and MAP-tau proteins are associated with stabilized (unchanging or non-plastic) cytoskeletal structures. Tau expression specifically is important for regulating the selective stabilization of microtubules accompanying extension of the neuronal cell membrane. Although phosphorylation of MAP2 itself is not sufficient to induce the formation of dendritic spines, the local expression of microtubule-associated proteins are useful indices of dynamic changes in dendritic surface area.

Rats were fed 8% (ad lib), 20% (pair-fed; equicaloric with 8% group), 20% (ad lib), or 50% (pair-fed; equicaloric with 8% group) casein for 36 weeks and their brains were collected. Selected areas of the brain (frontal cortex, entorhinal cortex, striatum, cerebellum) were dissected. MAP2 and MAP-tau proteins were solubilized and separated using 3-15% polyacrylamide gel electrophoresis. The blots were incubated with mouse monoclonal anti-MAP2 and mouse monoclonal anti-Tau antibodies. The results of the immunoblots are presented in the appendix (Figures 1 - 4). Both low (8%) and high (50%) casein diets resulted in increased expression of high molecular weight MAP2 (HMW-MAP2) in the frontal cortex and cerebellum, but diminished expression of HMW-MAP2 in the striatum and entorhinal cortex, compared to the equicaloric, 20% casein-fed group (Figures 1 and 2). Caloric restriction alone resulted in a dramatic increase in HMW-MAP2 in the striatum and entorhinal cortex, but a decrease in HMW-MAP2 in the cerebellum. In the striatum and cerebellum, these effects apparently were compensated when dietary protein was fed at both 8% and 50% levels. Caloric restriction had no effect on expression of HMW-MAP2 in the frontal cortex. Expression of MAP-tau proteins were not significantly altered by manipulation of dietary protein or caloric restriction (Figures 3 and 4).

Manipulating dietary protein and caloric restriction resulted in a complexed pattern of changes in MAP2 expression which must be interpreted cautiously. However, a few observations may be made from the data which are intrigueing:

1) Expression of HMW-MAP2 was sensitive to manipulations of dietary protein in all brain regions analyzed.

2) The frontal cortex was sensitive to manipulations in dietary protein, but apparently insensitive to caloric restriction.

3) Within the same brain region, high- and low-dietary protein resulted in qualitatively similar expressions of HMW-MAP2.

4) As a result of manipulating dietary protein, intracellular mechanisms associated with neuronal plasticity were enhanced in the frontal cortex and cerebellum, but diminished in the striatum and entorhinal cortex.

Project 14: Dietary protein and brain amino acid profiles (Jeffery Brock and Richard Tulley). High dietary protein in rats results in hyperactivity, hyper-responsiveness, anxiolysis, and increased basal arousal levels. These observations suggest a very complex pattern of neurotransmitter and neuromodulator involvement. First, hyperactivity and hyper-responsiveness suggest an over-expression of the central dopaminergic system in the brain. Interestingly, total dopamine content of the brain remains apparently unchanged by a 50% casein diet. However, analysis of discrete brain nuclei by punch dissection and HPLC/ED revealed that certain areas of the brain had significantly greater amounts of dopamine, while levels were diminished in other areas. Increases in dopamine should be accompanied by an increase in the availability of the amino acid, tyrosine, as precursor. Although others have shown that total brain tyrosine level is not significantly elevated by 50% casein diet, there remains the possibility that the differential increases in dopamine concentration may be accompanied by corresponding increases in tyrosine levels.

Hyper-responsiveness may involve under-expression of the serotonergic system. High dietary protein tends to reduce uptake of the amino acid tryptophan (serotonin precursor) into the brain. The observed hyper- and hypo-responsiveness in rats consuming high- and low-protein diets, respectively, are consistent with reports of decreased pain sensitivity in humans following tryptophan administration.

The phenomenon of anxiety, or aversion, behavior in rats is very complex. Serotonergic system involvement is complex and controversial. Also, high dietary protein results in anti-aversive, or anxiolytic, behavior which is similar to the action of diazepam in humans. Diazepam is an agonist for part of the gamma-aminobutyric acid (GABA) receptor complex. The implications are

that GABA activity may be inhibited in certain areas of the brain, as a result of the high-protein diet, in a way which dis-inhibits the expression of behavioral reflexes. To make the story even more complicated, there is compelling evidence that inhibitory amino acids (GABA, glycine, taurine) and excitatory amino acids (glutamate, aspartate) may function to counter-balance each other, within highly localized anatomical domains, to control the expression of behavioral subroutines.

Rats consuming a high-protein diet also display an increase in basal arousal level. The mechanism for this observation is not known, although the observed amplification of cortical negativity responses is consistent with dopamine over-expression. The phenomenon also may involve an increase in energy expenditure, which may be revealed by alterations in brain levels of amino acids associated with the tricarboxylic acid cycle (glutamate, alanine) and reflect changes in cerebral protein metabolism (leucine, methionine).

The conclusion from this discussion is that it is imperative to determine the effects of dietary protein on rat brain amino acid profiles in order to elucidate the mechanism of protein-induced hyperactivity, hyper-responsiveness, and anxiolysis. With this objective in mind, rats were fed 8%, 20%, or 50% casein diets for 4 weeks and for 16 weeks, then sacrificed by rapid decapitataion, and their brains stored at -80° C until ready for processing. Selected areas of the rats' brains (frontal cortex, parietal cortex, occipital cortex, enhorhinal cortex, cerebellar cortex, striatum, septum, hippocampus, anterior thalamus, and hypothalamus) were dissected and processed for analysis. Samples of each area were taken into 600 microliters of 3% perchloric acid, weighed, and sonicated. Protein assays are presently being performed on the homogenates. The samples will be filtered (.45 um) and amino acids will be analyzed, using precolumn derivatization and HPLC, by the PBRC Analytical Laboratory.

Project 15: Diet and Stress (Jeffery Brock). Data collection has begun on a new project which will investigate the effects of stress on cognitive function and the potential for nutritional intervention to protect against stress-related cognitive deficits. Rats will be subjected to rapid eye movement (REM) sleep deprivation for 96 hours to develop a model of stress-induced cognitive dysfunction.

After decades of sleep research, involving a variety of animals including humans, much is yet unknown about the physiologic role of rapid eye movement sleep. However, a few facts are established which are relevant to our purpose:

* Sleep deprivation has obvious effects on behavior, depending upon its duration (e.g., tiredness, time disorientation, visual misperceptions). The significance of behavioral and perceptual

deficits for human sleep function is unclear. They could be due to some form of conflict between a sleep drive and the need to stay awake, and/or some form of lack of cerebral restitution.

* Investigators agree that the cerebrum is the part of the body which is in the most need of sleep; perhaps for recovery of, and changes in, plastic processes (by comparison, the midbrain and brainstem structures appear not to require rest). For the cerebrum, sleep may be the means for its most efficient recovery following activity.

* In humans, the psychological performance tests which are most vulnerable to sleep deprivation are not complex decision-making tasks, but the simple, low-interest, long-duration tasks, such as 1 hour of auditory vigilance. Decrements in performance also occur during tasks which are regarded as "uninteresting" by the subject. The attribute of "interest" apparently evokes compensatory effort on the part of the subject during performance of the task (viz., modulation of preparatory arousal levels).

* In rats, sleep deprivation is an extremely potent stressor. For this species, prolonged sleep deprivation means imminent death. Rats survive only 11-32 days during total sleep deprivation; they survive only 16-54 days during REM sleep deprivation. Prior to death, rats experience pathologies with obvious multiple organ-system involvement. A short list of these abnormalities includes increased cerebral excitability, increased basal arousal, enhanced drive-related behaviors, increased food intake, increased energy expenditure, increased plasma norepinephrine, loss of weight, decreased body temperature, and severe ulcerations of the skin. Most of these observations are only correlated with sleep deprivation, with little certainty of causality. The precise cause of death in chronically sleep-deprived rats is unknown. More toward our purpose, mechanisms involved in the effects of sleep deprivation on the rat cerebrum remain a mystery, and are a central issue in this study.

The effects of 96-hour REM sleep deprivation (REMD) on higher brain function in the rat will be characterized using behavioral, neurophysiological, and biochemical methods. The specific experimental aims of this study are to determine the effects of 96-hour REMD on:

1) shuttlebox performance, using a fixed ratio-2 contingency, to demonstrate decrements in reference memory (this paradigm is very sensitive to disruption of frontal cortical function). A more standard shuttlebox paradigm would probably be insufficient. Running is an important part of the rat's defense behavior, and largely reflexive. Thus, the cognitive consequences of foot-shock in the REM-deprived animal may be masked in a simple shuttle/escape task. However, when the task is associatively more difficult, or motorically more demanding (such as the fixed ratio-2 contingency,

or the 3-second delay paradigm), subtle deficits in performance are revealed.

2) rat swimming/immobility test, a rather simple but important evaluation of the rat's adaptability or ability to cope with sudden stress. This test is selectively sensitive to REM sleep deprivation.

3) electrical correlates of behavior; recording cortical negativity responses (a purely frontal cortex-generated potential), to demonstrate abnormalities in preparatory arousal and selective attention; sensory mismatch negativity responses (an associative cortex-generated potential), to demonstrate decrements in working memory (the duration of an auditory memory trace). Also, effort will be make to record P300 responses in the rat. The P300 potential is known to be generated by the septal-hippocampal circuit, a part of the forebrain intimately involved in memory storage and retrieval, and which mediates attentional-switching in response to environmental cues.

4) macronutrient selection, using the Three-Choice diet (carbohydrate, protein, fat), to demonstrate abnormalities in food intake. In rats, macronutrient selection is normally well-regulated, and mediated largely at the level of the anterior and ventromedial nuclei of the hypothalamus by a complex interaction of neurotransmitters, neuropeptides, and amino acids. Identifying abnormalities in macronutrient selection in the sleep-deprived rats will contribute to our understanding of why the animals increase their total intake, yet lose weight to a debilitating degree.

5) neuronal dendrite morphology (dendritic spine density), to demonstrate plastic changes in key areas of the brain (cerebrum, septum, hippocampus, striatum) which may contribute to impairment of cognitive function. It is now known that dendritic spines play an important role in neuronal function, and abnormalities in dendritic spine density are correlated with learning, general states of alertness, and mentation.

6) monoamine neurotransmitter and amino acid levels in selected areas of the brain, to determine the nature and extent of neurochemical changes which may contribute to observed functional abnormalities. Our lab has shown that monoamine neurotransmitter activity is sensitive to dietary manipulation and the effects of diet on amino acid profiles in the blood and brain are already well known. Amino acid levels are especially important to measure, since they are not only precursors for most classical neurotransmitters, but some amino acids (glutamate, aspartate, GABA, taurine, glycine) are themselves putative neurotransmitters. Other amino acids are related to the tricarboxylic acid cycle (e.g., glutamate and alanine) and reflect changes in cerebral protein metabolism (e.g., leucine and methionine).

After the animal model for REMD has been established, studies will be undertaken to determine if nutritional manipulation can sustain performance under REMD conditions. Characterizing the effects of REMD using this multi-disciplinary approach should not only clarify mechanisms underlying REMD-induced cognitive deficits, but reveal possible points of intervention where we may protect against those deficits.

At present, our laboratory awaits completion of reconstruction of the behavioral testing laboratories. Meanwhile, we have initiated control studies for one of the electrical correlates of behavior, the sensory mismatch negativity (MMN). We have successfully recorded MMN responses from urethane/alpha-chloralose anesthetized rats. The data represents not only the first recording of MMNs from the rat, but the first such recording in any anesthetized animal. Additional experiments are planned which employ relevant manipulations that will validate the interpretation of MMN responses as a measurement of the duration of auditory memory traces. The recording of sensory mismatch negativity in anesthetized rats presents an economical model for studying the mechanisms of short-term memory formation. This data should be of interest to a broad spectrum of Neuroscientists who are generally interested in the study of higher brain function.

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IV. Fort Polk Heart Smart Project

Introduction

During the mid-1980's, the U.S. Congress felt the need to examine issues of nutrition and general health status among military personnel and their dependents. This resulted in a congressional mandate to the U.S. Army in 1987 to devote funds to research in these issues. A portion of this funding went to a team of researchers under Dr. Gerald Berenson. Tasked to gather descriptive data and to implement a health promotion program, the team chose Fort Polk, Louisiana as its research site.

Fort Polk is the current home of the 5th Mechanized Division and has a contingent of about 15,000 active duty personnel (see Table 1, in Appendix). It represents about 8,000 - 9,000 military families and about 6,000 - 8,000 child dependents. As such, Fort Polk was of sufficient size to conduct the required research.

Accomplishments

Over the course of two years of data collection (Summer, 1989 - Summer, 1991) the Fort Polk Heart Smart Project accomplished the following:

1. Nutritional, Lifestyle, and Physical Activity Assessments of 200 Military Wives

2. Cardiovascular Disease (CVD) Risk Factor Screening of over 700 Soldiers, Their Spouses, and Their Children

3. The Development of Health Promotion Materials for Military Families

4. The Implementation of a Health Promotion Program in about 70 Military Families

Goals and Project Design

As implied above, four basic goals were developed for research at Fort Polk. These were:

To describe typical eating, activity, and other lifestyle traits of military dependents.

To develop data on levels of CVD risk factors in military families.

To develop appropriate materials and techniques for military family health promotion programs.

To test such materials on military families.

Fort Polk Substudies

Research at Fort Polk was divided into three projects:

Project 1 - Assessment of Dietary Intake and Physical Activity in Military Wives

A sample of 200 wives intact military families underwent a battery of nutritional and other assessments to establish routine eating, activity, and other behavioral patterns (Table 2 in Appendix). A CVD risk factor screening was also offered.

Project 2 - Cardiovascular Risk Assessment of Military Families

A sample of about 125 intact military families received a CVD risk factor screening including blood pressure, anthropometric, lipid, and lifestyle questionnaire determinations (Table 3 in appendix).

Project 3 - Family Health Promotion

A program for family health promotion, including prudent eating, exercise, stress and smoking reduction was developed and implemented on about 70 intact military families (Table 4 in Appendix). Participants received CVD risk screenings, dietary and activity recall, health history, and psycho-social questionnaire evaluations on a pre- and post-implementation basis.

Results

Nutritional, demographic, socio-economic (SES), and sociological data on Project 1 wives and their families are included in Attachment 2-A, Appendix.

Demographic and SES data are presented first. Race distribution data show the sample to be about 2/3 white, 1/5 black, less than 1/10 Hispanic or other. Husbands' rank shows whites to predominate at the E-1 to E-4 and also the 0-1 to 0-4 level, while E-5 to E-9 and WO-1 and WO-2 are slightly over-represented among blacks. About 80% of the sample has a high school diploma or some college education. Median family size was 4. About 10% of the wives participated in the federal Woman, Infant, Child (WIC) program, while 1.5% received food stamps. Other data are available on number of cars and television sets owned per family.

Dietary information begins with family meal and snacking patterns. Families in this sample typically eat one meal per day together and wives enjoy 1-3 episodes of between-meal snacking on a typical day. Families also usually eat four or more meals per week while watching television.

Monthly food consumption practices show milk, bread, and margarine to be the most frequently eaten food items with organ meats and veal to be most infrequently used. Other frequently consumed items include ketchup, chips, candy, cheese, eggs, hot dogs, poultry, and cereal. After margarine, mayonnaise and cooking oil are the most frequent sources of fat and oil.

Monthly grocery expenditures range between $100 and $250 for about 2/3 of the families. Less than 10% of this group spends $350 or more while less than 5% spends less than $100. About half of these families spend $23 - $50 per month eating out.

Most families (about 60%) eat fried food regularly. Whole milk is somewhat preferred over 2% and skim.

About 2/3 of the Project 1 women report desiring to lose weight. Most want to lose between 5 and 20 pounds, but about 20% wish to lose in excess of 30 pounds.

The balance of nutrition data depict nutrient intake, adherence to recommended daily allowances (RDA), and comparability of Fort Polk results to other surveys. Overall, these women consume between about 1800 and 2000 kcals per day, with about 35% of kcals coming from fat (12-13% from saturated fat). Black women report the lowest daily caloric intake (1771 kcals). Wives of enlisted men report high intakes (2101 kcals).

Generally, the women in this sample report intake between 40 and 70% of RDA for most vitamins and minerals. Those figures are slightly better for the B-complex vitamins.

Comparisons with other surveys show Fort Polk women to be intermediate in caloric consumption and low in percent of calories from fat. Reported carbohydrate intake is relatively high at Fort Polk with cholesterol intake relatively low.

CVD Risk Factor Data

(Note: This portion of the final report replicates the findings presented in the 1990-1991 annual report. In the interest of keeping attachment material to a minimum, the appropriate tables and figures are to be found as Attachments A-C in the 1991 Annual Report and may be obtained by request.

Attachment A of the 1991 Annual Report contains summary figures and tables for the CV risk factor and lifestyle questionnaire evaluations completed on Fort Polk personnel and their dependents. Representative figures and tables from Attachment A of the 1991 Annual Report are appended and are designated Attachment A-1 and A-2. Table A-1 outlines the age, race, and sex characteristics of this group. 703 individuals participated in CV risk screening ending in January 1991. Adult females predominated reflecting

their sole participation in Project 1. In the subsequent descriptions, comparisons are made with Bogalusa Heart Study norms on an age-appropriate basis. Racial comparisons are limited to black/white contrasts.

Attachment A of the 1991 Annual Report presents anthropometric and body composition results for this group. Overall, the Fort Polk sample shows little racial difference in height or in comparison with the Bogalusa population. Weight shows systematic contrasts within Fort Polk females, with adult blacks being systematically heavier. There is also a tendency for the Fort Polk adults to be heavier than their Bogalusa counterparts.

Measures of obesity show typical racial contrasts. By all gauges, skinfold and Quetelet (body mass) index (wt/ht^2) black women exceed white ones. White men demonstrate thicker skinfold measures than black men, but similar Quetelet figures. Upper quartile of Quetelet index (greater than 27.0) includes 40-50% of whites at Fort Polk and 40-70% of blacks depending on age and sex.

Blood pressure data are presented in Attachment A of the 1991 Annual Report. As is seen nationally, black adults at Fort Polk show somewhat higher levels of both systolic and diastolic measures than whites. Pressures, however, are systematically lower when compared to Bogalusa norms.

Lipid data are presented in Attachment A of the 1991 Annual Report. Generally, black men exhibit higher levels of both total cholesterol and low-density lipoprotein (LDL) while no clear trend is evident in women. In addition, black men at Fort Polk show higher values relative to Bogalusa norms. High-density lipoprotein (HDL) levels are higher in black males than in white ones, while no clear pattern emerges among females at Fort Polk. Comparisons with Bogalusa results show little contrast among whites but do show consistently higher levels in Bogalusa blacks. Very-low-density lipoprotein tends to be systematically higher in whites of both sexes.

Approximately 40-50% of Fort Polk men of both races exceed NCEP guidelines for moderate elevation of total cholesterol (greater than 200 mg/dl). Black men additionally show 10-20% with high levels of cholesterol (greater than 239 mg/dl). Women fall in the moderately elevated range about 30-40% of the time with, typically, 5-10% falling in the high grouping. All race-sex groups, except white males, at Fort Polk exceed their Bogalusa counterparts for excessive levels of cholesterol. LDL results mirror those of total cholesterol, as expected.

A figure of 34 mg/dl or less of HDL is a potential risk category for heart disease. Fort Polk screenees show little tendency in this direction, with fewer than 5% of the sample of either sex, overall, falling into this assignment. By comparison, Bogalusa

residents fall below 35 mg/dl at a rate 2-4 times greater than Fort Polk adults.

Blood chemistry data are presented in the Annual Report 1990-91. Overall, blacks tend to exceed whites in levels of all proteins and related molecules, LDH, calcium, phosphorus, and creatinine. Whites exceed blacks in levels of urea and uric acid. No consistent trends are evident in other measures.

Hematology results reveal, as is usually seen, that whites exceed blacks in levels of hemoglobin, hematocrit, and white blood cell (WBC) count.

The final section of Attachment A of the 1991 Annual Report is amended as Attachment A-2 and presents data on lifestyle influencing variables including smoking behavior, alcohol consumption, socio-economic (SES) indicators (military rank, educational level, etc.), and family size. Smoking behavior statistics indicate that about between 36 and 47% of adult males and about 30% of adult females classify themselves as smokers. All race/sex groups except white males, who are much higher, approximate National Health Survey norms in this respect. Alcohol consumption is reported on a regular basis by between 40 and 70% of adult respondents. Blacks report a higher prevalence of drinking than other races. Overall reported alcohol consumption tends to be higher at Fort Polk though is typical of the military world-wide and is consistent across ranks in men.

Family Health Promotion

The Family Health Promotion (Project 3) component of the Fort Polk Heart Smart Project was initiated with a pilot study on 6 families during the summer of 1990. The study began on a full scale in September of 1990. Three cycles of program administration occurred between that time and July 1991. Approximately 70 families, comprising about 225 individuals, participated.

The practical goal of this sub-study was to develop a self-contained health education and promotion model suitable for military families and to deliver this model to test for acceptability, efficacy, and its effect on CVD risk factor levels and behaviors.

Attachment B of the 1991 Annual Report contains the resulting Health Promotion Manual. It is available on request. It comprises a statement on background, rationale, and theoretical base and then proceeds through the mechanisms of delivering such a program at a military installation.

The actual program consisted of a CVD risk factor screening and a battery of health-related questionnaires delivered on both a pre- and post-test basis and a set of educational sessions deliverable

in a 8-12 week time frame. The manual describes session topics, agendas, and ancillary activities as well as incentive and maintenance programs. Calendars of actual sessions are also presented.

Attachment C of the 1991 Annual Report contains the complete battery of Project 3 evaluations and is available on request. Included are: standards and normative data, CVD risk factor screening data forms, lifestyle questionnaires, nutritional assessments, and process/program evaluations. Ancillary subject communication forms (screening feedback, consent letters, etc.) are included.

The data collection phase for Project 3 ended on July 25, 1991. Data are entering the final edit stage and, excepting dietary recall information, will be available for analysis in August, 1991. Nutritional assessments require product and menu research and are scheduled for final analysis during the fall of 1991.

Conclusions

Research at Fort Polk has served to increase the descriptive database for CVD and related biomedical traits in service personnel. In addition, it has extended these observations to military dependents. Such information will prove valuable for future policy formation.

Comparisons with various standards demonstrate overall similarity of military families with American society as a whole. This indicates general nutritional and at least short-term, health status adequacy. It also implies similar susceptibility to long-term chronic or other conditions such as heart disease, diabetes, and certain cancers. In addition military families face environmental stresses unusual in civilian settings. Families must contend with regular absences of parents, who may spend more than six months of a year off-post attending regular military training functions. Moreover, as we saw twice during our research period, war may unpredictability remove family members for long periods with no certainty of return. The emotional fall-out from these responsibilities can be enormous.

These considerations make military families prime candidates for general health and wellness promotion programs. Preventive medicine both on the strictly biological and emotional levels offers great potential savings. These should be evident in reduced costs for medical attention and improved productivity in service personnel.

The Fort Polk Heart Smart Project has developed a model for family health promotion which addresses those issues. It is in manual form, transferable to a number of settings. It may be administered via a variety of military institutions; military

hospitals, health promotion committees, volunteer groups, etc. It is well received by families and can be modified to serve current needs. Its adoption by the Army would well augment current health promotion activities available at military installations.

V. Menu Modification Study

Introduction and Background

Since 1985, nutrition initiatives have been introduced into the Armed Forces Recipe Service, the Army Master Menu and the Army Food Service Program to provide soldiers with diets lower in sodium, fat, and cholesterol. The Military Nutrition Division of the United States Army Research Institute of Environmental Medicine (USARIEM) has conducted assessments of soldiers' nutrient intakes. These studies resulted in the following nutrition related recommendations: continue revision of the Armed Forces Recipe File to reduce sodium in recipes, continue to decrease the percentage of calories obtained from fat to 35% or less of total calories, and provide soldiers low cholesterol, low fat alternatives to eggs, and evaluate the acceptability and impact of using this approach to moderate soldiers' cholesterol intakes.

The Menu Modification Project incorporates modification of two weeks of Army garrison menus to meet the nutrition targets specified by the Army. The purpose of the Menu Modification Project is to provide healthful, nutritious menu selections which moderate soldier's sodium, fat, and cholesterol intakes.

Progress

The Army Menu Modification Project began in January, 1990. Human subjects review approval was obtained from the Louisiana State University, Baton Rouge campus Committee on the Use of Humans and Animals as Research Subjects, the Human Use Review and Regulatory Affairs Office of the Surgeon General, U.S. Army, and the LSU Medical Center Institutional Review Board.

During the first year of the project, three part-time student workers were hired and trained to prepare menu items for taste panel testing. Recruitment, selection, orientation and training of nine volunteer taste panel participants was completed. A graduate assistant was hired to monitor preparation, service, and evaluation of approved modified menu items in the LSU athletes' dining facility. A total of 69 items were prepared and evaluated by the taste panel. Eighteen food formulations were prepared in quantity, served and evaluated for acceptability by the athletes in Broussard Cafeteria. Taste panel and athletes' scores can be found in Appendix V of the 1990 Annual Report.

Analysis of Army and modified recipes was carried out using The Extended Table of Nutrient Values (ETNV). Initially, Army recipes

were modified and analyzed. Later, a full day's menu as served by the Army was modified and comparisons made between the regular and modified menus. While striking differences were not seen in initial experiments, trends were noted. Fat was lowered from 42.5 to 39% of calories. Reduction of eggs at breakfast resulted in a decrease of 814 mg to 450 mg of cholesterol. Unfortunately, sodium content of the modified menu was not lowered. Comprehensive analyses of the initial experiments are included in Appendix VI of the 1990 Annual Report.

During the second year of the project, sixteen recipes were prepared in quantity and served to athletes dining in Broussard Cafeteria on the Louisiana State University campus. Students rated the items for acceptability. Quantity preparation of recipes was conducted in Broussard Cafeteria. The results of the acceptability ratings are also found in the Ninth Quarterly Report.

During the Tenth Quarter, five days of menus were modified and analyzed using the Extended Table of Nutrient Values (ETNV). The data was presented to Army officials at the time of their December 13, 1990 visit to the Pennington Center. The following conclusions were drawn from the data presented (see Tenth Quarterly Report):

1. Modifications resulted in a decrease in fat from 40% to 36% of calories and in saturated fat from 12% to 10% of calories.

2. Carbohydrate represented 48% of calories in the modified menu and 45% in the regular menu.

3. In terms of calories, protein was only 1% higher in the modified menu (16% vs. 15%).

4. There was a significant reduction in calories (12%) from a mean of 3500 to 3080 per day.

5. Cholesterol was significantly reduced (36%) from a mean of 720 mg to 462 mg per day. A comparison of breakfast menus also showed a significant reduction in cholesterol.

In January, 1991, a meeting was held at USARIEM to review the progress and plans for the Menu Modification Project. The results of this meeting were as follows:

1. The original two week menu cycle from the Army 1989 Master Menu will be replaced with a more current version from the 1991 Army Master Menu.

2. The fat content of the menu revision will be modified from 35% of total kilocalories from fat to more closely approach 30% of total kilocalories from fat. Individual recipe items will be reformulated as needed to more closely approach the lowered level of fat.

3. A nutrient analysis of the two weeks of Army menus will be calculated using all selections offered on the menu, an individual analysis of each menu items and a two week analysis of the average both the regular and modified menus.

4. Acceptability testing by student athletes will be discontinued due to lack of an adequate number of participants.

5. Quantities for 100 portions will be checked.

In February 1991, Dr. Catherine Champagne (Johnson) presented a paper entitled "Computer Analysis of Army Recipes and Menus Using the Extended Table of Nutrient Values (ETNV)" at the annual meeting of the Southern Association of Agricultural Scientists in Fort Worth, Texas. An abstract of this paper can be found in the Ninth Quarterly Report.

Coding and analysis of Army and modified menus has continued. Two hundred and sixty recipes were submitted to Nutrient Data Systems at the Pennington Biomedical Research Center for analysis using the Extended Table of Nutrient Values. Quantity recipe testing was continued until the end of the spring 1991 academic semester in a student cafeteria on the Louisiana State University campus. Coding and nutrient analysis will continue and plans will be formalized for small batch acceptability testing of reformulated recipes. Quantity preparation will resume in the fall 1991 semester and continue through the spring 1992 academic semester.

The analyses of 101 modified and regular Army recipes can be found in the appendix of the Twelfth Quarterly Report.

Conclusions

To achieve the nutrition related recommendations set by USARIEM, it is apparent that further work needs to be done in the area of menu modification of Army menus. Initial data from the analysis of regular Army menus revealed the fact that fat, cholesterol and sodium are significantly higher than desirable. From our menu alterations, it was evident that improving breakfast menus led to the most significant reduction in fat and cholesterol in soldiers' diets. More work in modifying other meals will help to achieve our objectives of reducing total fat, sodium, and cholesterol. While it is evident that reducing sodium may be a more difficult task, additional work should be devoted to this project. Implementing the testing of modified menus at an actual garrison, such as Fort Polk, could serve to more effectively test acceptability of modified recipes. With Pennington's involvement and the use of our ETNV data base, the Menu Modification Project could be expanded to become an efficient mechanism by which to help achieve USARIEM's nutrition recommendations of lowered fat, cholesterol, and sodium.

APPENDIX

I. Clinical Research

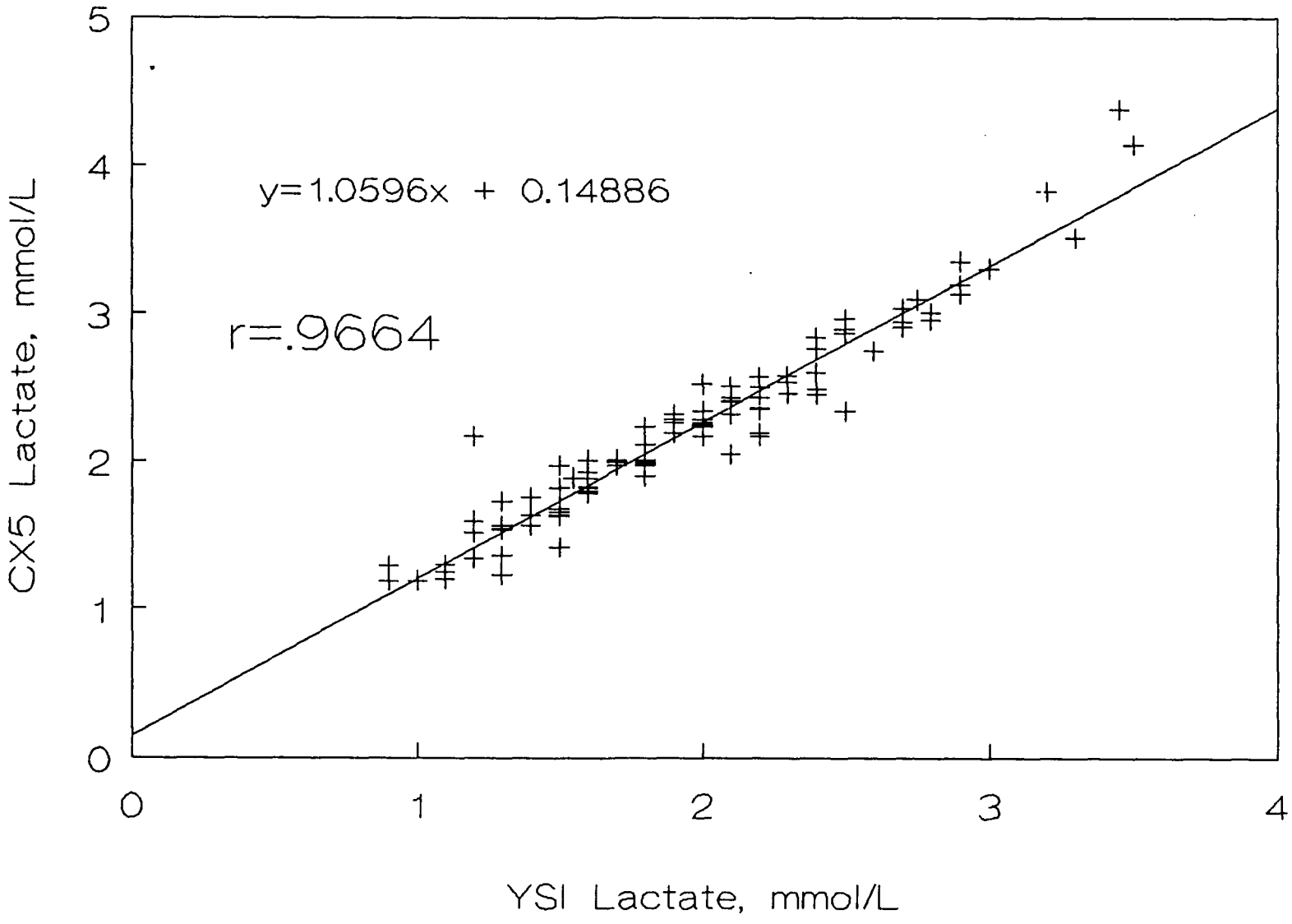
II. Fort Polk Heart Smart Project

Tables
Attachment A-1
Attachment A-2

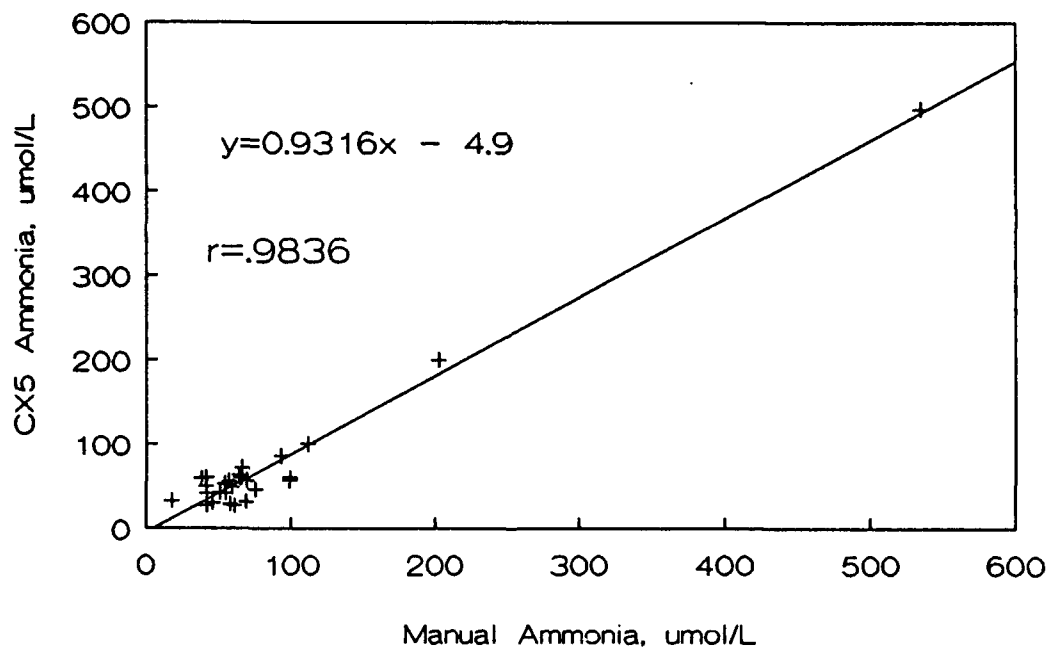
CLINICAL RESEARCH APPENDIX

Lactate Correlation

CX5 vs YSI Lactate Analyzer

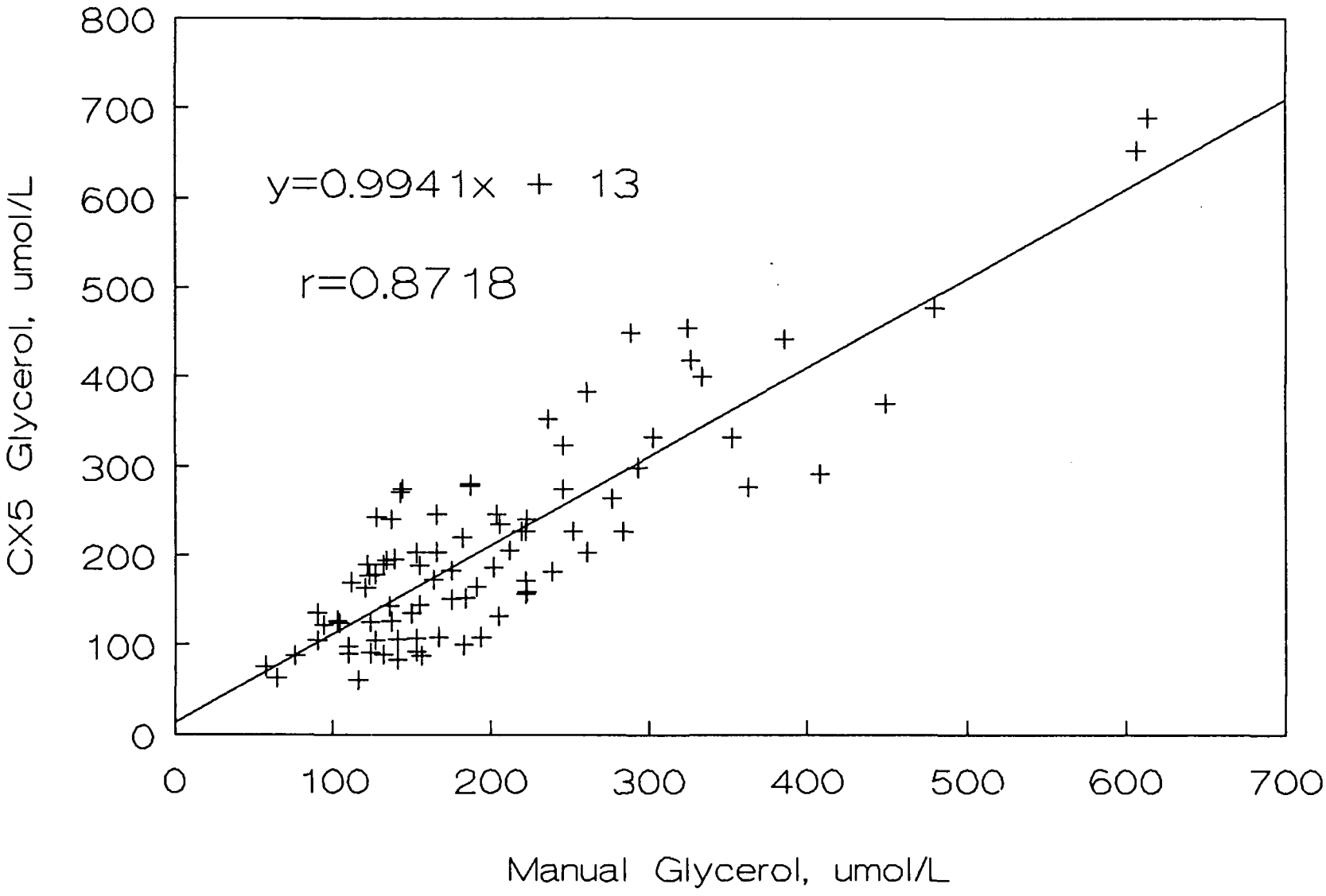


Ammonia Correlation
CX5 vs Manual

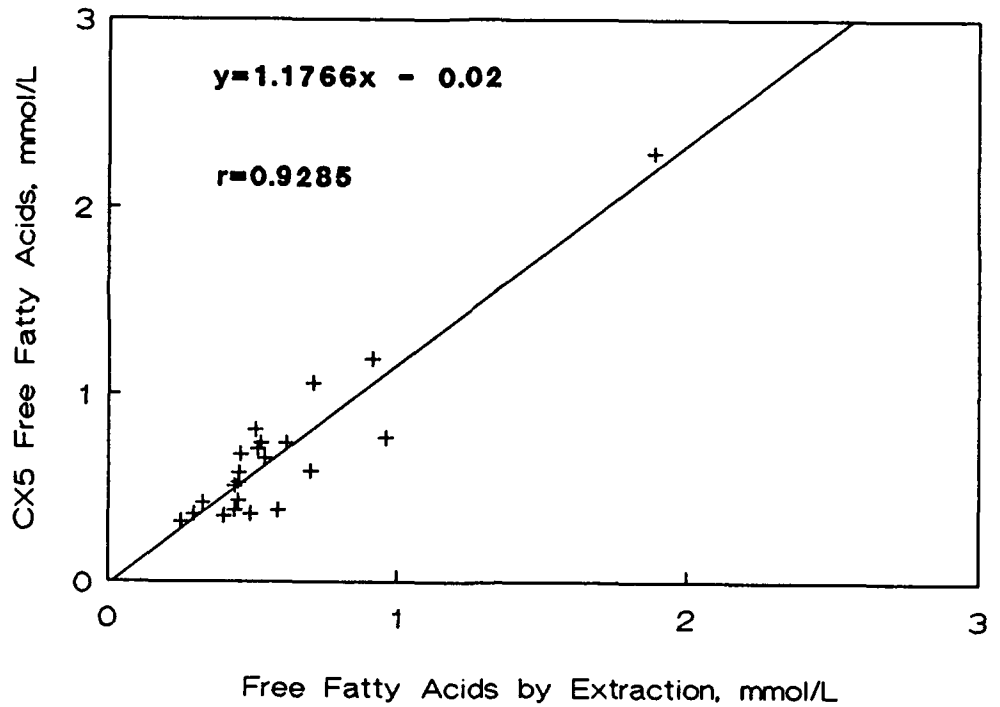


Glycerol Correlation

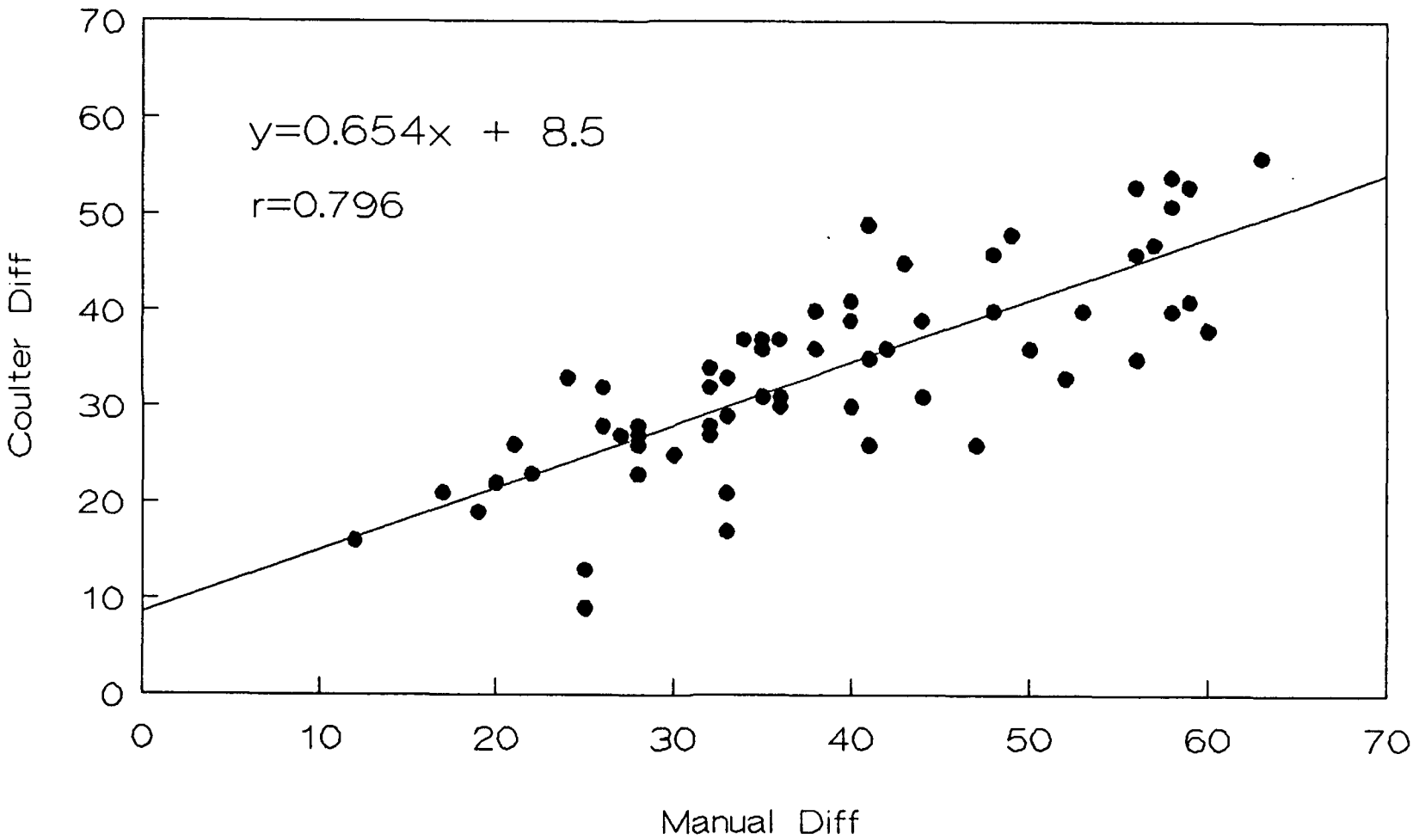
CX5 vs Manual



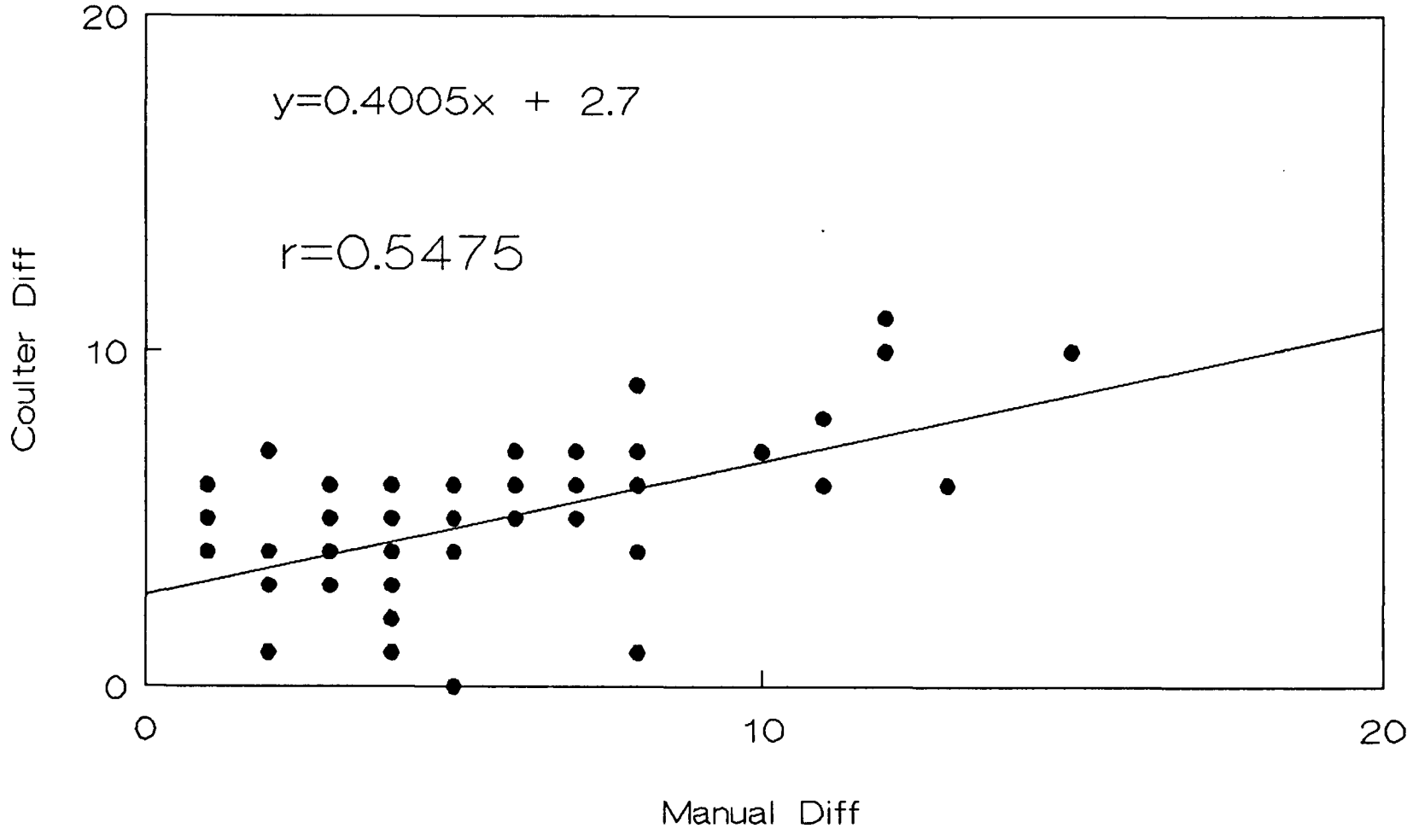
Free Fatty Acid Correlation
CX5 vs Copper Extraction



Coulter STKS
Lymphocytes

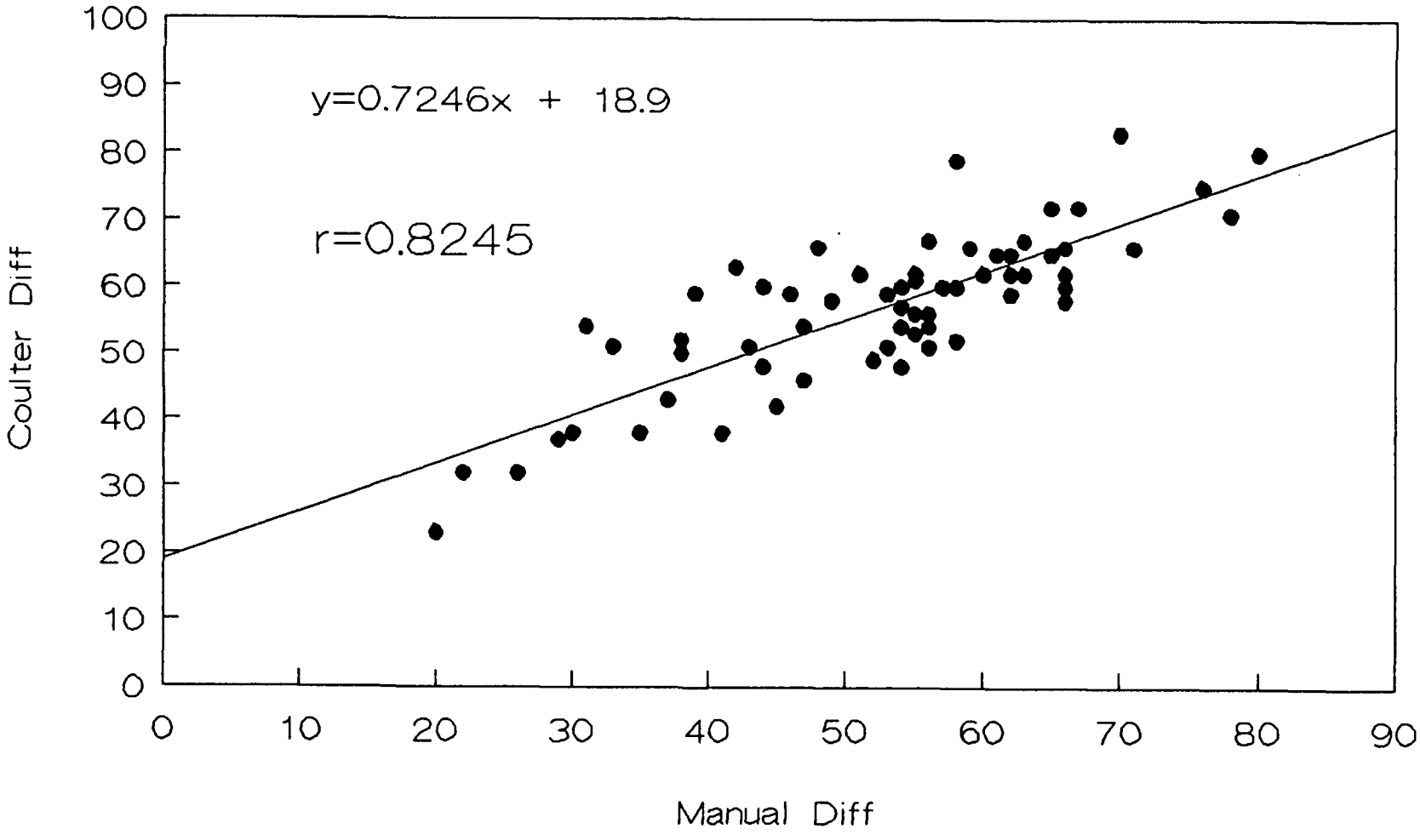


Coulter STKS
Monocytes



Coulter STKS

Neutrophils



BASO	
COULTER	MANUAL
3	1
4	0
1	0
0	1
1	0
1	0
1	2
0	1
1	0
1	0
1	0
2	0
1	0
1	0
3	0
0	1
2	0
1	1
2	0
1	0
1	0
1	0
1	0
1	0
1	0
1	0
0	1
0	1
3	0
1	1
2	0
1	0
1	0
1	0
1	0
1	1
2	0
5	0
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2	0
2	0
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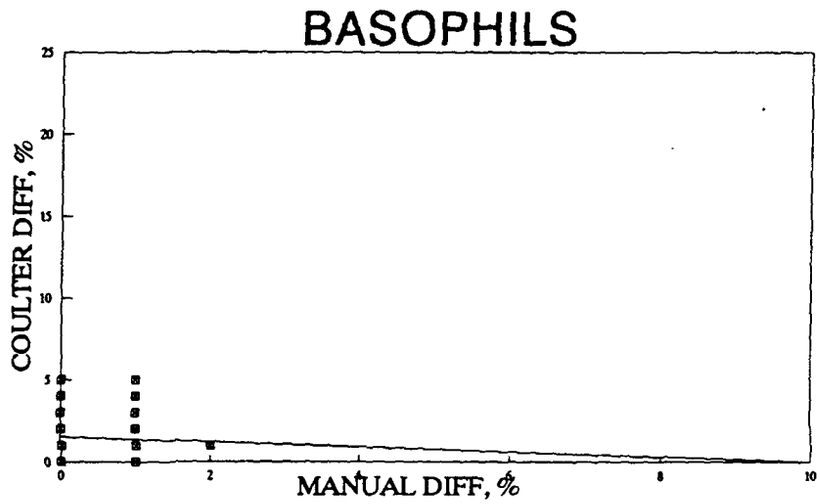
1	0
2	0
2	1
3	0
1	2
1	0
1	1
2	1
1	0
1	1
1	1
4	1
1	0
1	1
2	0
2	0
1	0
1	0
1	1
1	1
1	1

Constant	Regression Output:	1.53550296	
Std Err of Y Est		1.00383496	
R Squared		0.02049448	0.14315892
No. of Observations		79	
Degrees of Freedom		77	
X Coefficient(s)		−0.2514793	
Std Err of Coef.		0.19812619	

0	1.53550296
2	1.03254438



BASO

COULTER	MANUAL
5	1
1	1
1	0
1	1
1	1
3	1
1	0
2	1
3	0
1	1
1	1
0	0
1	1
1	1

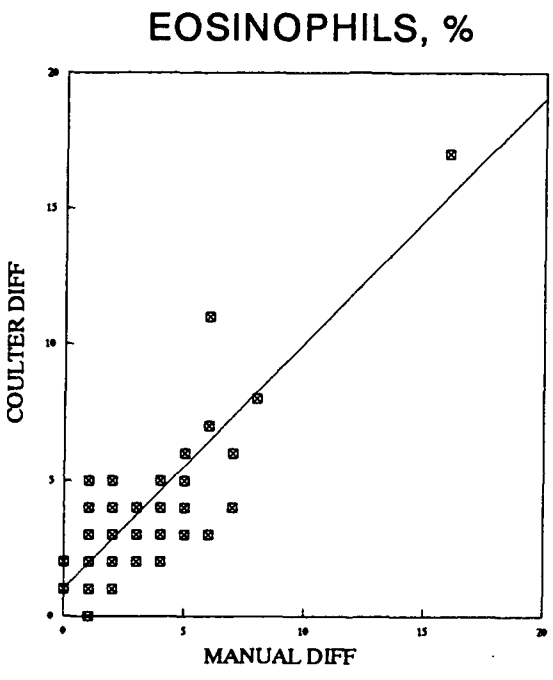
EOS

COULTER	MANUAL
1	2
6	5
1	1
8	8
4	3
1	1
3	1
3	6
2	2
4	5
3	2
5	4
2	1
2	2
3	3
17	16
4	3
4	2
3	1
4	1
2	1
4	5
3	4
2	1
1	1
1	2
1	0
4	4
3	3
5	5
5	2
2	0
3	2
1	1
3	4
0	1
2	4
5	1
3	2
4	5
3	4
2	0
2	1
4	3
1	0
2	2
3	4
2	1
2	1
3	1
3	4
2	2
1	2
1	0
1	2
4	7
4	4
2	1
2	3
2	3
2	2
3	2
2	0
4	4
2	1

Regression Output:

Constant	0.916	
Std Err of Y Est	1.3221	
R Squared	0.6568	0.8104
No. of Observations	91	
Degrees of Freedom	89	
X Coefficient(s)	0.81	
Std Err of Coef.	0.0621	

0	0.916
10	9.0161



EOS

COULTER	MANUAL
3	2
1	0
6	7
2	2
7	6
2	1
2	2
4	4
1	2
2	3
5	5
1	2
5	1
11	6
2	2
3	3
4	3
3	5
3	2
3	2
3	2
1	2
2	2
4	3
4	2
4	2

PENNINGTON BIOMEDICAL RESEARCH CENTER

Clinical Research Laboratory

Temperature Log

Refrig:	std White
Freezer:	

Range: 2 – 8 °C

Year:	1990											
Month:	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Day												
1			4 RT		2 RT	5 JZ		4 JZ		5 RT	6 RT	
2			3 RT	4 RT	3 RT		4 LM	4 RT		6 RT	5 RT	
3				6 RT	3 RT		5 LM	4 JZ		6 MD		5 mm
4				5 Adj RT	4 RT	5 JZ				5 JZ		5 mm
5		3 RT		3 JZ		4 RT	6 RT		5 LM	5 RT	5 MD	5 mm
6		3 RT		0 JZ Adj		5 JZ	5 JZ	4 JZ			5 RT	5 mm
7		2	4 KM		5 RT	5 RT		4 JZ	5 JZ		6 RT	5 mm
8		3 RT	4 KM		5 RT	5 JZ		4 JZ		5 MD	5 RT	
9		3 RT	3 KM	2 RT	4 12T		6 KB KM	4 JZ		5 RT	6 MD	
10				2 RT	5 RT		6 LM		5 RT	5 JZ		4 RT
11				2 RT	6 RT	6 JZ			5 RT	5 RT		
12		2 RT	4 RT	4 RT	6 JZ	6 JZ	6 LM		4 RT	5 RT	6 RT	
13		0 Adjusted RT 2	3 RT	H		4 JZ	6 LM	4 JZ	4 RT		6 MD	
14		3 RT	4 RT	0		3 JZ		5 RT	5 JZ		6 JZ	
15		3 RT	3 RT	1	5° KM	4 RT		5 RT		5 LM	6 RT	
16		2 RT	3 RT	2			5 LM	4 RT		6 MD	6 MD	
17				4 RT	6 JZ		6 RT	4 RT	5 RT	5 RT		
18				3 RT	6 JZ	4 RT	5 RT		5 RT	4 RT		
19		3 RT	4 RT	3 RT			5 RT		5 RT	5 MD	6 JZ	
20		3 RT	3 RT	2 JZ	6 RT	4 JZ	10 KB	6 JZ	5 RT		6 MD	
21		4 RT	3 RT		5 RT	4 JZ		6 JZ	5 JZ		5 JZ	
22		3 RT	2 RT		6 JZ	5 JZ		3 RT		6 MD		
23		3 RT	4 RT	2 RT	7 RT		5 LM	4 RT		5 RT		
24				4 RT	5 RT		6 LM	5 JZ	5 RT	5 RT		
25				5 RT	5 RT		5 LM		6 RT	6 MD		
26		3 RT	4 RT	5 RT		6 RT	5 LM		5 JZ	5 RT	4 RT	
27		Mardi Gras	4 RT	5 JZ		5 RT	4 KB	4 RT	5 RT		5 RT	
28		4 RT	4 RT		5 JZ	5 JZ		4 RT	5 RT		6 JZ	
29	3° RT		4 JZ		5 JZ	6 JZ		5 JZ		6 MD	6 MD	
30	2° RT		3 RT	2 RT	5 JZ		6 JZ	4 RT		6 RT	8 JZ	
31	2° RT		Blood	X	5 JZ		4 RT	4 RT		5 RT		

HOLIDAY

PENNINGTON BIOMEDICAL RESEARCH CENTER
Clinical Research Laboratory
DI Water Checks
Resistivity

DI Unit:	B
Type I: >10 Mohms	

Year:	1990											
Month:	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Day												
1						17.7 SZ		17.8 SZ		17.5 RT	17.6 RT	
2							17.7 [illegible]	[illegible] 17.8		17.6 RT	17.8 RT	
3							17.7 [illegible]	17.8 SZ		17.5 [illegible]		17.6 mm
4						17.7 SZ	OFF			17.8 SZ	.	17.8 mm
5						17.6 RT	18.2 RT		17.7 [illegible]	17.7 RT	17.8 mm	17.8 mm
6						17.8 SZ	17.8 SZ	17.8 SZ			17.8 RT	17.8 mm
7						17.5 RT		17.8 SZ	17.7 SZ		17.6 RT	17.7 RT
8						17.7 SZ		17.8 SZ		17.7 [illegible]	17.6 RT	
9							17.6 [illegible]	17.8 SZ		17.6 RT	17.7 [illegible]	
10							17.6 [illegible]		17.7 RT	17.8 SZ		17.6 RT
11						17.7 SB	17.6 RT		17.7 RT	17.6 RT		
12						17.8 SB	17.6 [illegible]		17.7 SZ	17.7 RT	17.7 RT	
13						17.8 SB	17.6 [illegible]	17.9 SZ	17.7 RT		17.7 RT	
14						17.9 SB		17.8 RT	17.8 SZ		17.8 SZ	
15						17.8 RT		18.3 RT		17.7 [illegible]	17.7 RT	
16							17.7 [illegible]	17.7 RT		17.7 [illegible]	17.7 RT	
17							17.8 RT	17.6 RT	17.7 RT	17.6 RT		
18						17.6 RT	17.8 RT		17.5 RT	17.6 RT		
19							17.6 RT		17.6 RT	17.6 [illegible]	17.8 SZ	
20						17.8 SB	17.8 [illegible]	17.8 SB	17.6 RT		17.8 mm	
21						17.7 SB		17.8 SB	17.8 SZ		17.8 SZ	
22						17.8 SB		17.8 RT		17.8 [illegible]	H	
23							17.7 [illegible]	17.8 RT		17.6 RT	I	
24							17.7 [illegible]	17.8 SZ	17.8 RT	17.8 RT	G	
25							17.6 [illegible]		17.8 RT	17.6 mm	H	
26						17.7 RT	17.7 [illegible]		17.8 SZ	17.6 RT	17.7 RT	
27						17.7 RT	17.7 [illegible]	17.9 RT	17.6 RT		17.6 RT	
28						17.7 SZ		17.7 RT	17.7 RT		17.7 SZ	
29						17.8 SZ		17.8 SZ		17.7 [illegible]	17.7 mm	
30						17.7 M	17.8 SZ	17.9 RT		17.7 [illegible]	17.7 SZ	
31							17.7 [illegible]	17.8 RT		17.7 RT		

PENNINGTON BIOMEDICAL RESEARCH CENTER
Clinical Research Laboratory

Reagent/Chemical Log

Reagent/Chemical:	CHOL (Beckman)

					QC 1		QC 2		
Date Rec	Amt Rec	Lot No.	Exp Date	Date Used	Value	Range	Value	Range	
11-30-89	2	M903060	3-31-90	3-1-90	124	124-132	76	76-82	RT
2-6-90	8	M906273	7-31-90	6-5-90	131	124-132	81	76-82	K
6-11-90	10	M911056	11-30-90	8-31-90	129	123-134	79	76-82	K
10-11-90	4	M002091	2-28-91	12-5-90	129	124-133	80	76-82	K

PENNINGTON BIOMEDICAL RESEARCH CENTER

Clinical Research Laboratory

Automatic Pipet/Measuring Device Check

Device:	200-1000 MLA Adjustable (13) (RIA)

Date	Measured Volume	% Error	% CV	Comments	Initial
4-18-90	(200) 200.6	0.32%	1.2%	27%E Hon rpt'd - OK	JZ
"	(1000) 1014.63	1.46%	0.47%		JZ
10-19-90	(200) 201.5	0.75%	0.59%		MO
10-19-90	(1000) 989.47	1.05%	0.53%		MO

FORT POLK HEART SMART PROJECT APPENDIX

Table 1

FORT POLK HEART STUDY

FORT POLK, LOUISIANA

5TH MECHANIZED DIVISION

15,000 Active Duty Personnel

10,000 Personnel With Depdenents

6,000 Child Dependents

Table 2

FORT POLK HEART STUDY

Project 1 - Baseline Assessment of Dietary Intake and Physical Activity in Military Dependents

Sample - 200 Wives of Military Personnel With At Least 1 Child

Goals - Characterize Eating, Food Purchasing, and Physical Activity Patterns

Measures

1. 24-Hour Dietary Recall
2. Food Purchasing Questionnaire
3. Pantry Survey
4. Physical Activity Recall
5. Health Habits Questionnaire
6. CVD Risk Factor Screening

Table 3

FORT POLK HEART STUDY

Project 2 - Cardiovascular Risk Assessment of Families at Fort Polk

Sample - 100+ Complete Families of Fort Polk Personnel

Goals - Establish Norms for CVD Risk Factors

Measures

1. Blood Pressure
2. Blood Lipids
3. Anthropometry
4. Medical History Questionnaire
5. Health Habits Questionnaire

Table 4

FORT POLK HEART STUDY

Project 3 - Family Health Promotion

Sample - 60 Complete Families of Fort Polk Personnel

Goals - Develop a Heart Health Education Model For Military Families

Measures and Procedures

1. CVD Risk Factor Screening
2. Eating, Physical Activity, and Behavior Modification Counseling
3. Health Habits Questionnaire

FORT POLK HEART SMART PROGRAM - ATTACHMENT A-1

FINAL REPORT - AUGUST, 1991

TABLE A-1

NUMBER OF SUBJECTS EXAMINED
FORT POLK HEART SMART PROGRAM

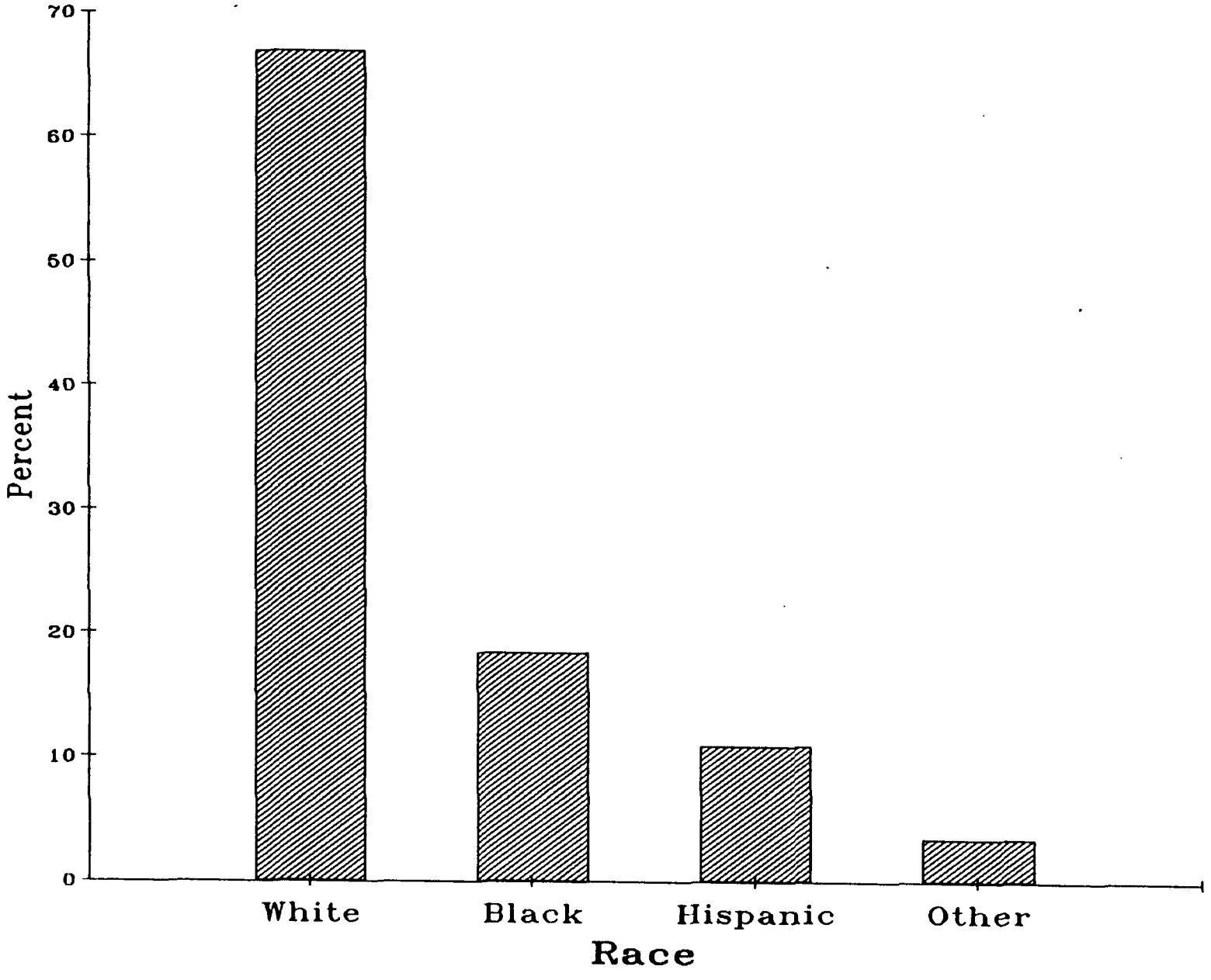
AGE

	0-9	10-19	20-29	30-39	40+	TOTAL
White Males	29	30	53	56	9	177
White Females	20	28	128	79	20	275
Black Males	9	10	25	21	5	70
Black Females	7	14	39	32	5	97
Hispanic Males	2	0	6	6	0	14
Hispanic Females	2	1	17	12	0	32
Other Males	3	4	2	0	1	10
Other Females	4	6	8	9	1	28
Total Males	43	44	86	83	15	271
Total Females	33	49	191	132	26	432
TOTAL	76	93	277	215	41	703

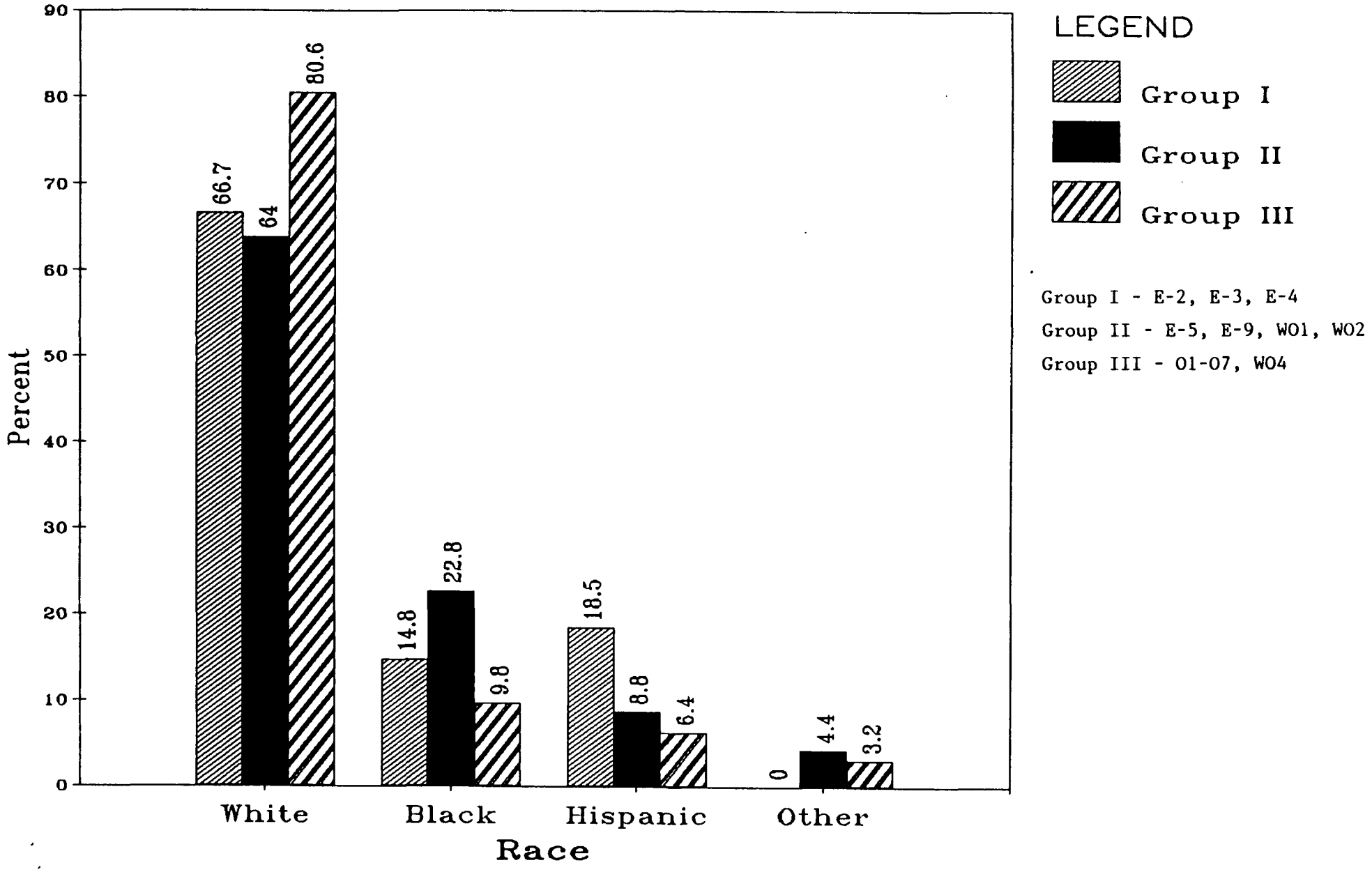
FORT POLK HEART SMART PROGRAM - ATTACHMENT A-2

FINAL REPORT - AUGUST, 1991

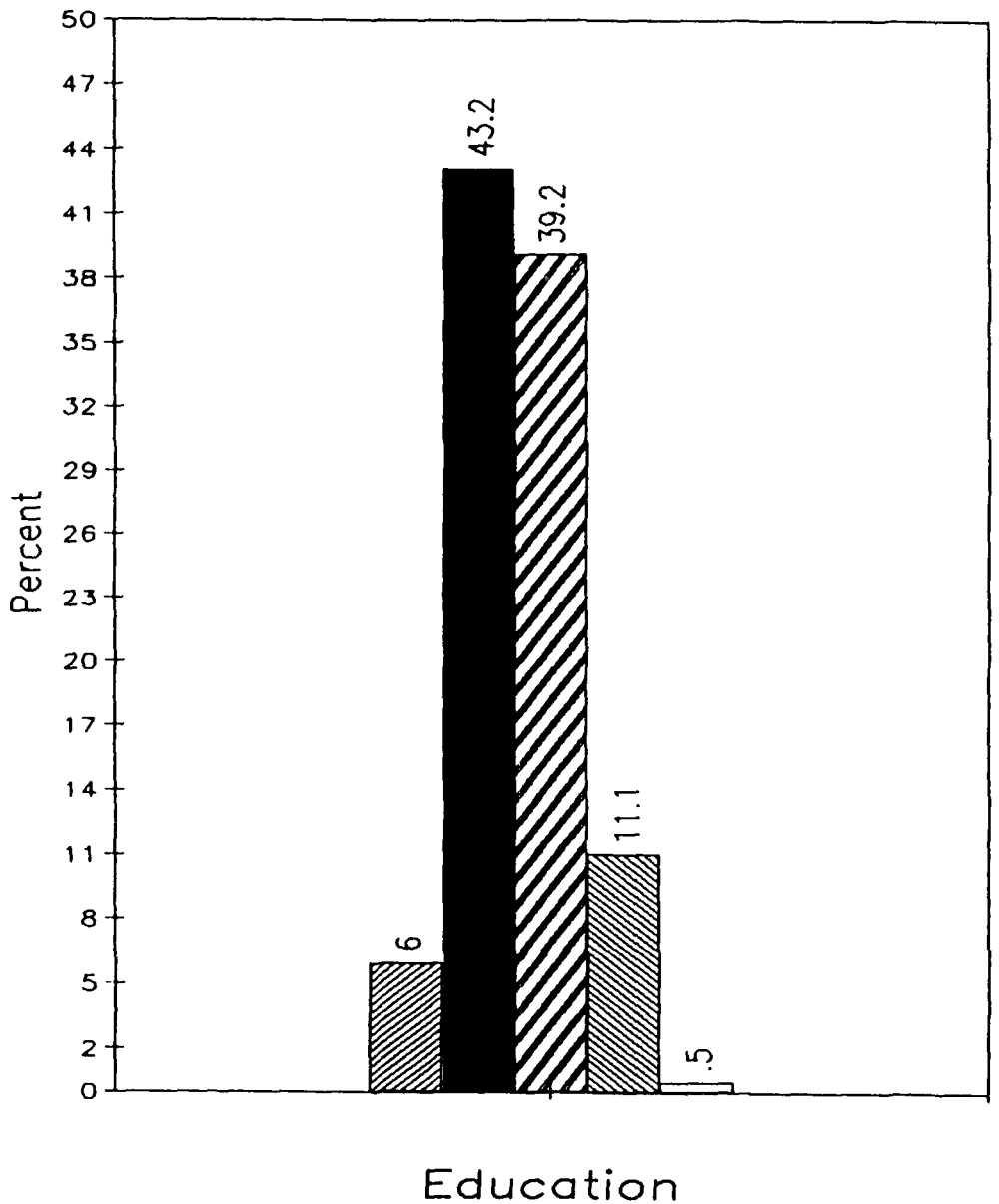
Race Distribution Among Military Wives
Fort Polk Heart Smart Program, 1989



Rank and Race of Husbands
Fort Polk Heart Smart Program, 1989



Education of Fort Polk Spouses
Fort Polk Heart Smart Program, 1989

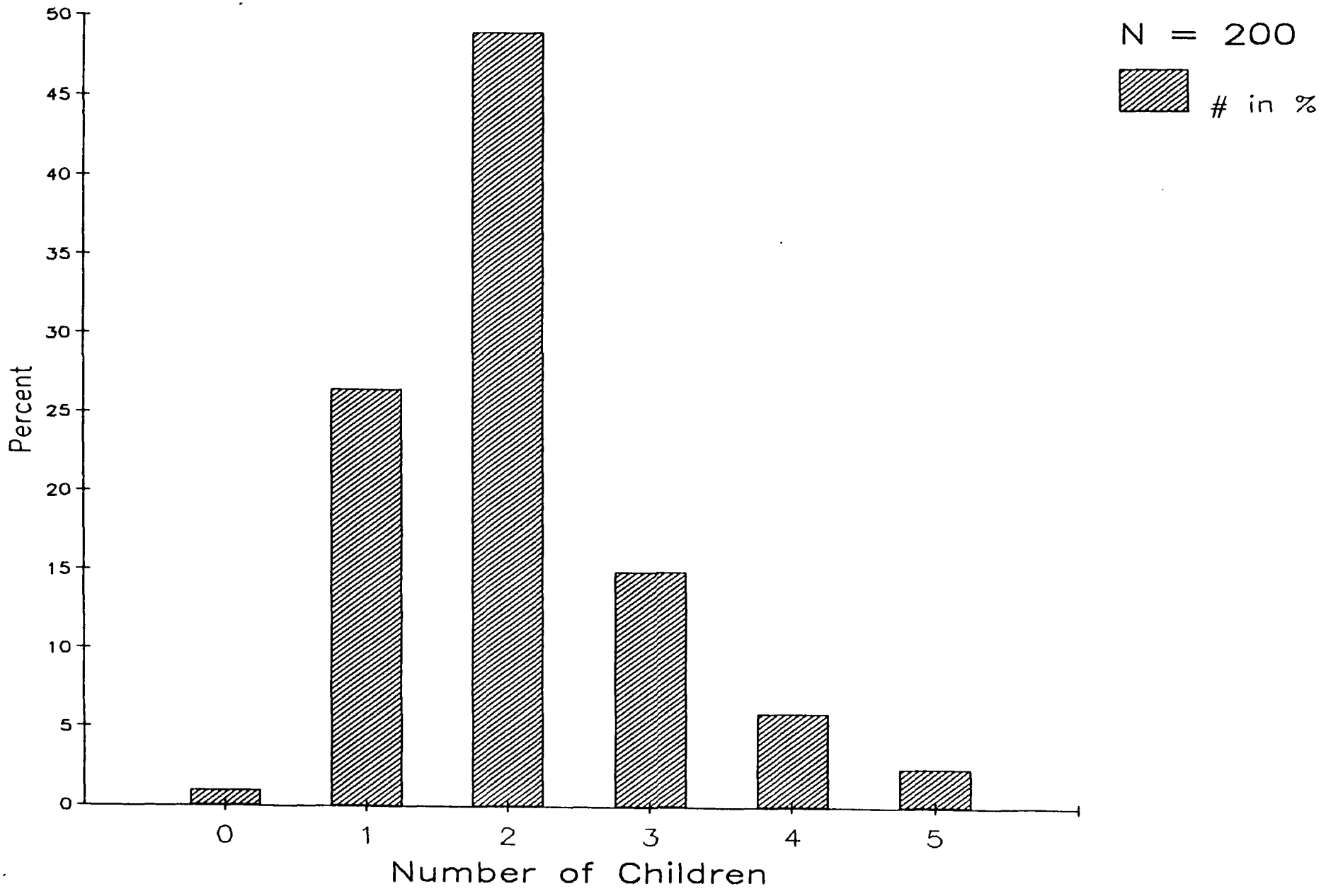


LEGEND

- Non High School Graduate
- High School Graduate
- Some College or Trade School
- College Degree
- Advanced Degree

N=199

Children of Military Wives
Fort Polk Heart Smart Program, 1989



Distribution of Husband's Rank for WIC and Food Stamp Users
Fort Polk Heart Smart Program, 1989

WIC

Rank	N	%
E-1	0	0
E-2	0	0
E-3	3	1.51
E-4	10	5.03
E-5	5	2.51
E-6	3	1.51
Total	21	10.55

Food Stamps

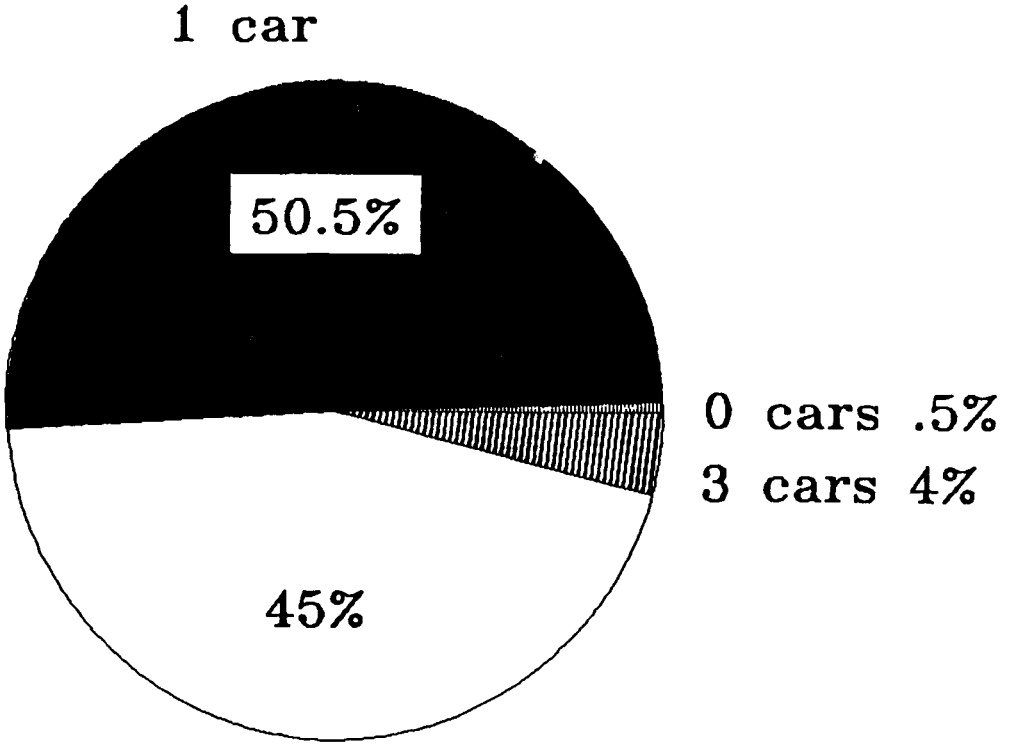
Rank	N	%
E-1	0	0
E-2	0	0
E-3	1	0.5
E-4	2	1.01
E-5	0	0
E-6	0	0
Total	3	1.51

Frequency Distribution for WIC and Food Stamps
Fort Polk Heart Smart Program, 1989

	WIC	
	N	%
Yes	21	10.5
No	179	89.5

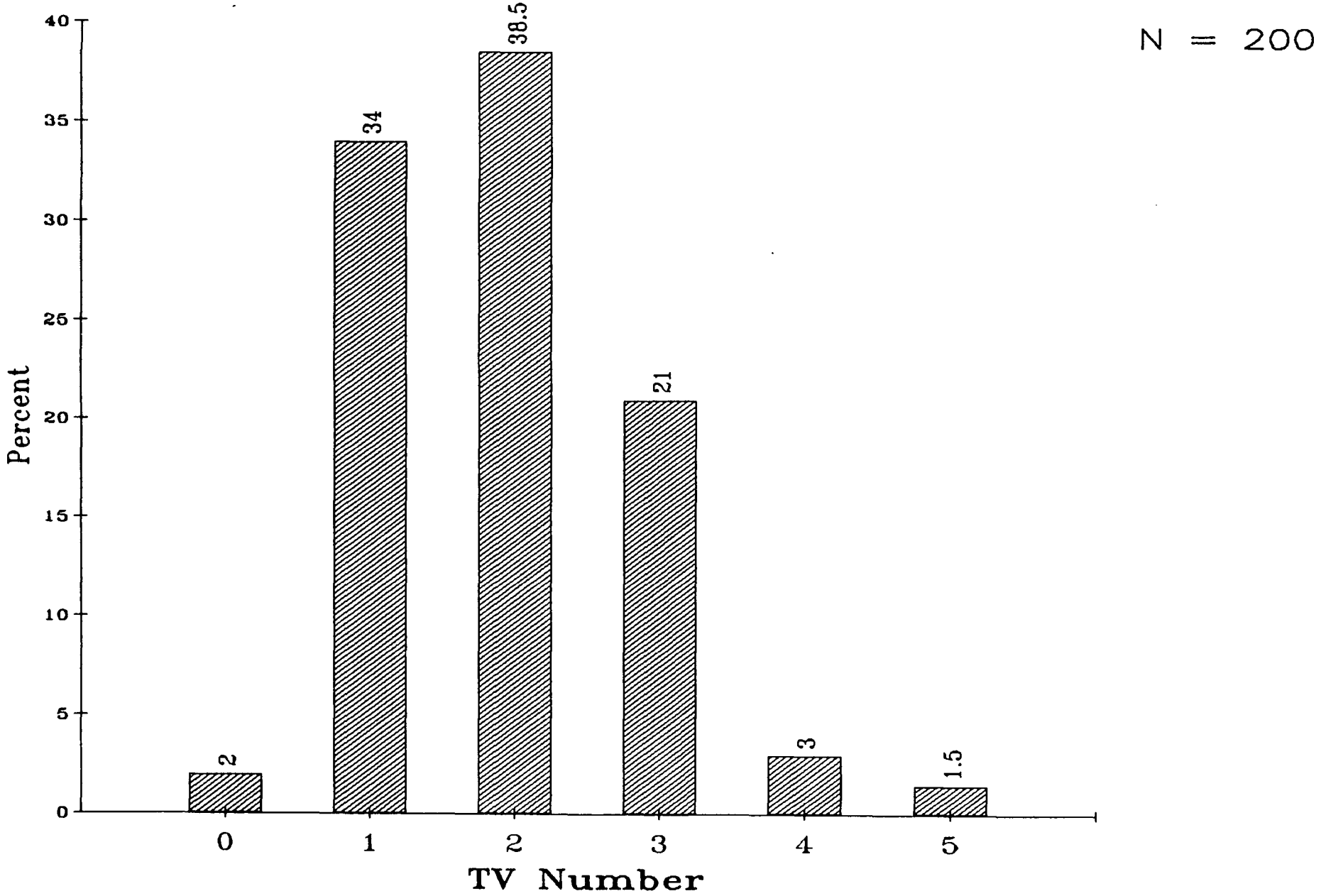
	Food Stamps	
	N	%
Yes	3	1.5
No	197	98.5

Number of Cars Per Family
Fort Polk Heart Smart Program, 1989

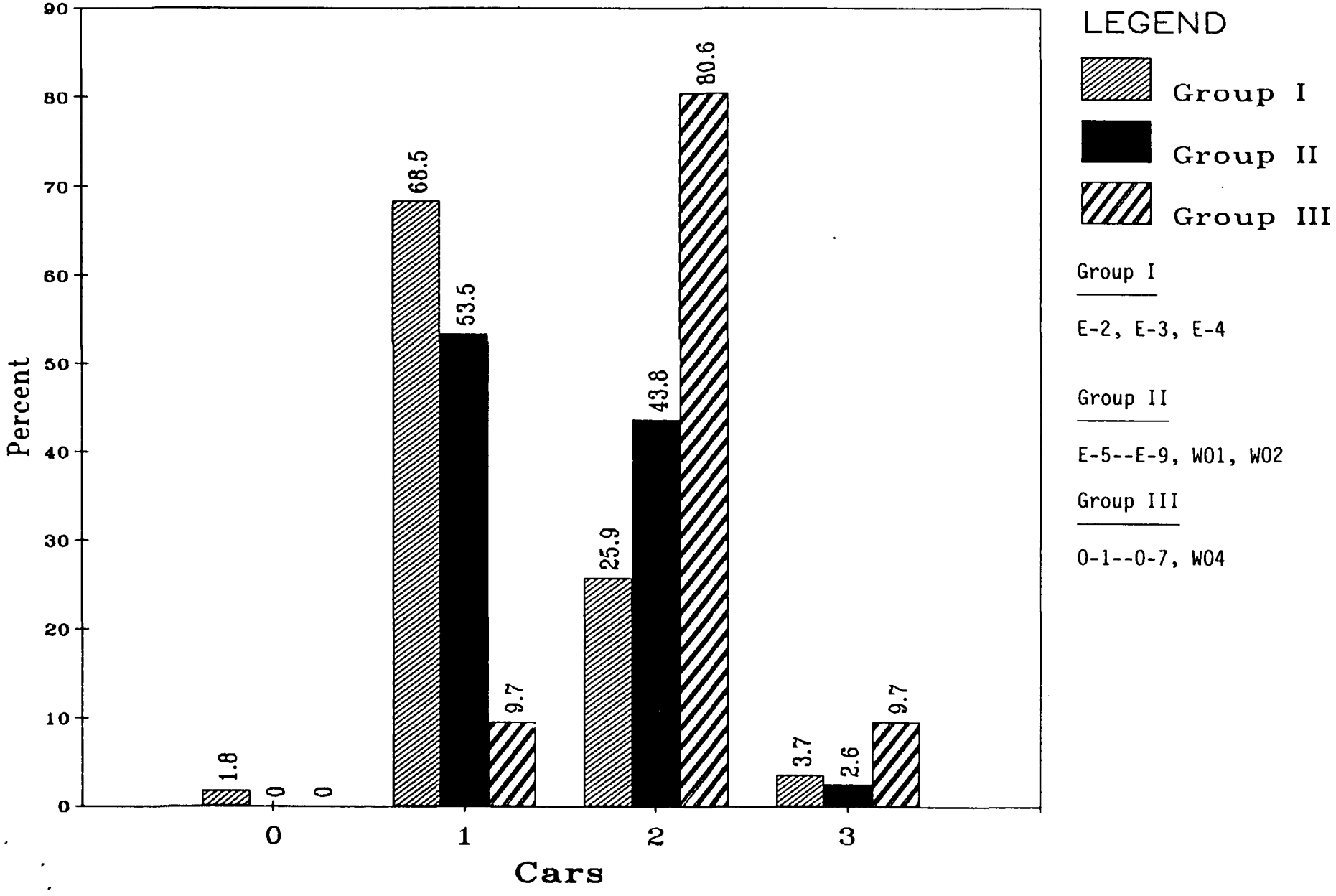


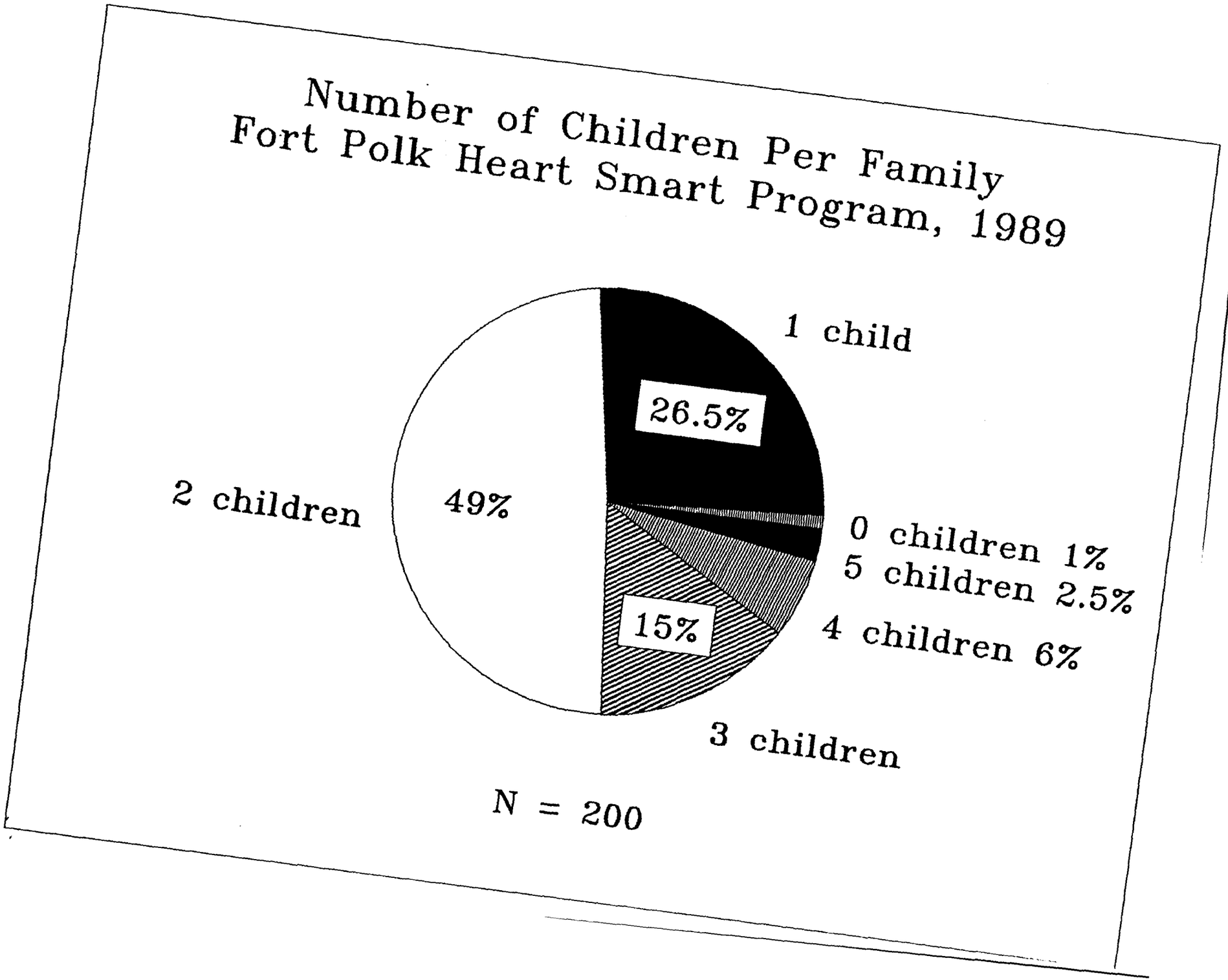
N = 200

Number of Televisions Per Family
Fort Polk Heart Smart Program, 1989



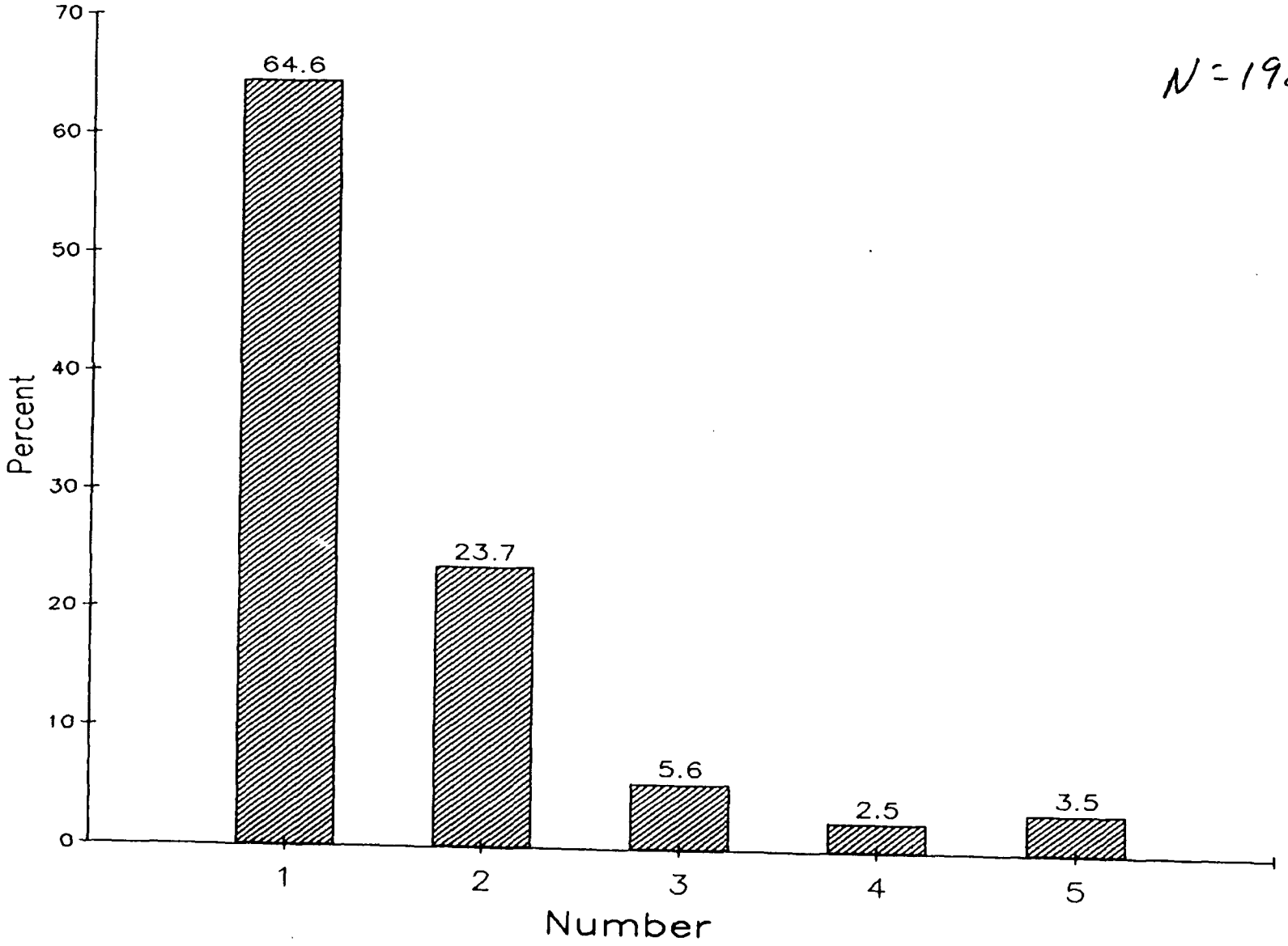
Ranks of Husbands and Number of Cars Owned
Fort Polk Heart Smart Program, 1989



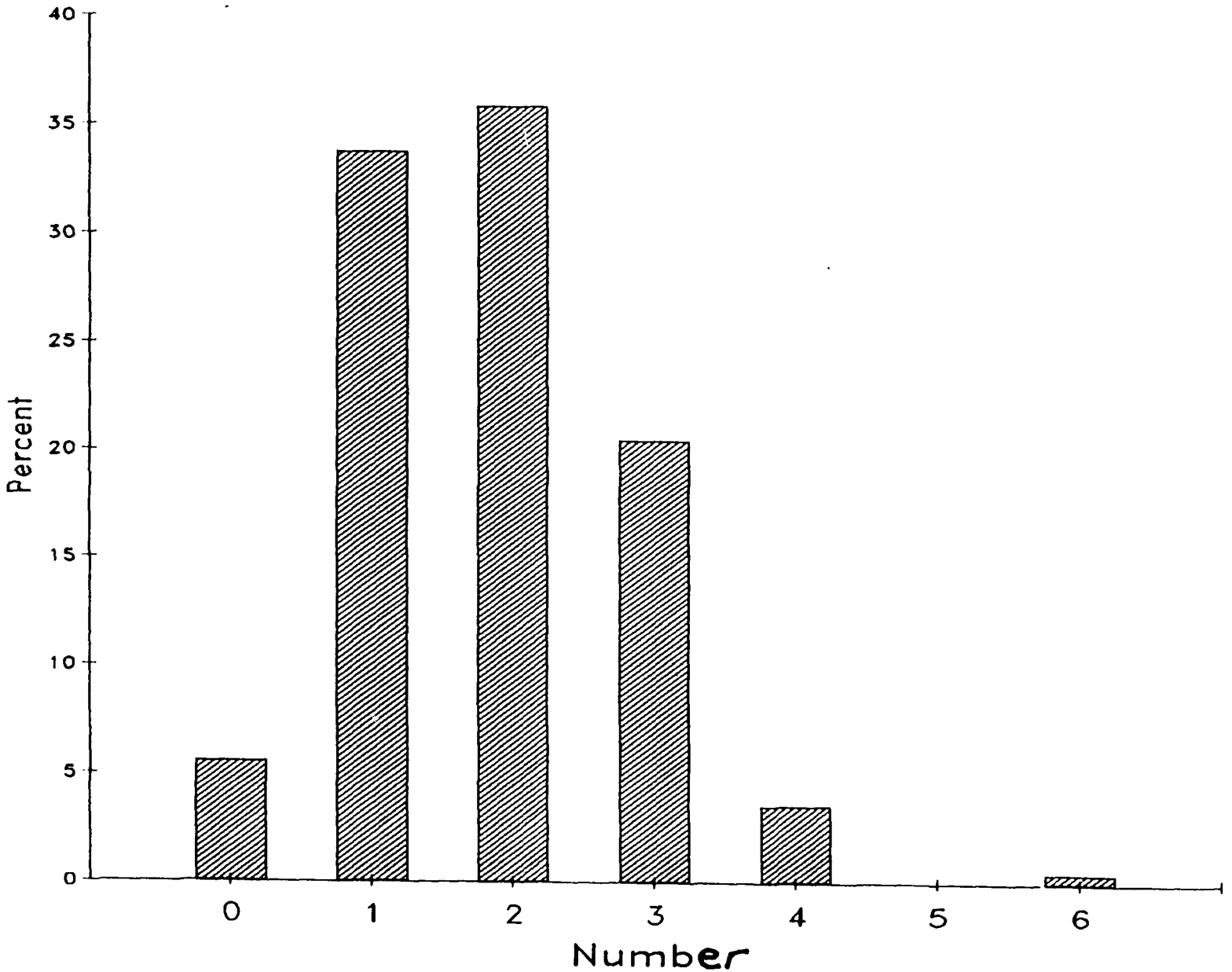

Number of Children Per Family
Fort Polk Heart Smart Program, 1989
1 child
26.5%
2 children
49%
0 children 1%
5 children 2.5%
15%
4 children 6%
3 children
N = 200

Family Meals Eaten Together Per Day
Fort Polk Heart Smart Project, 1989

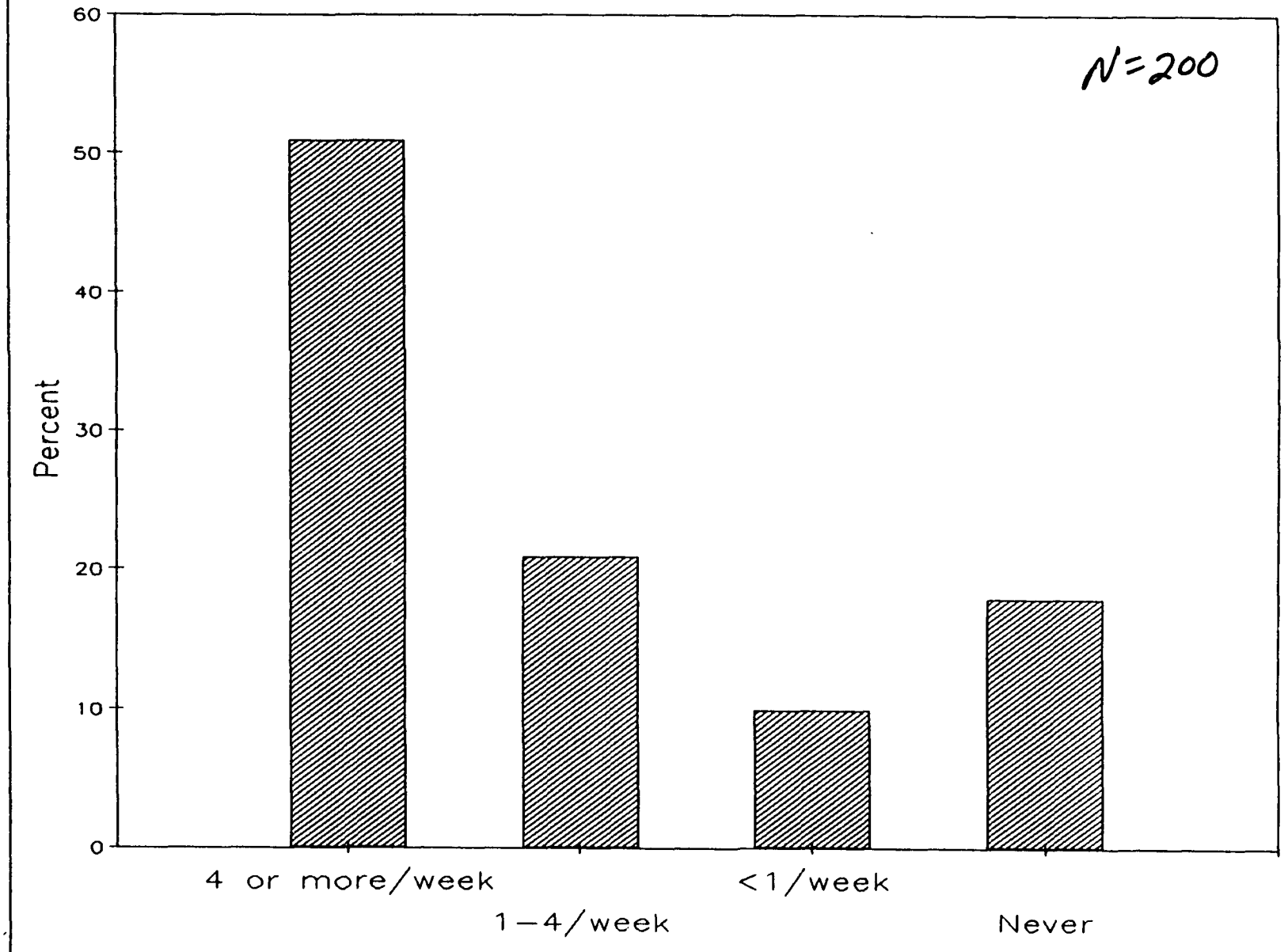
N = 198



Number of Snacks Eaten Per Day
Fort Polk Heart Smart Program, 1989



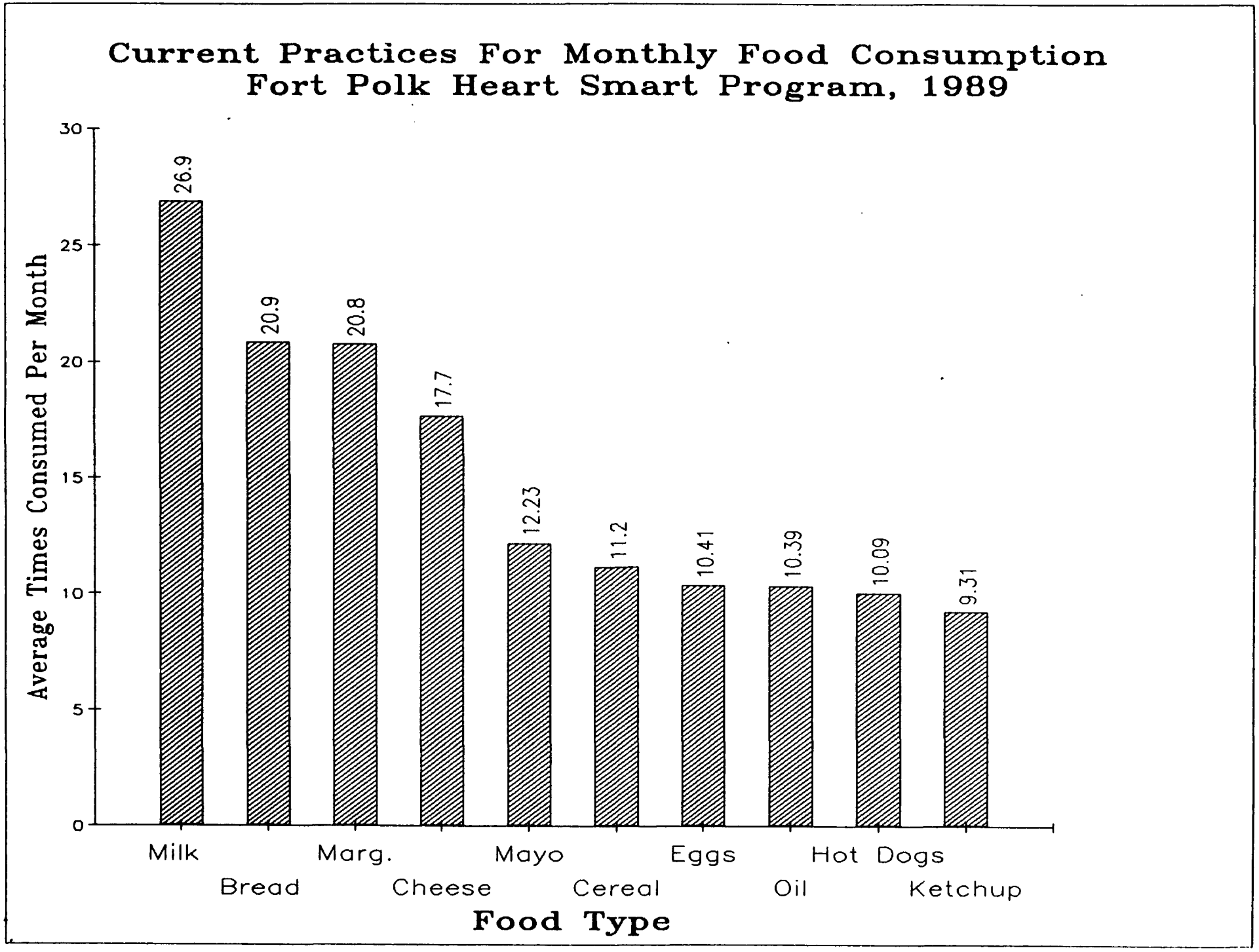
Meals Eaten While Watching TV
Fort Polk Heart Smart Program, 1989

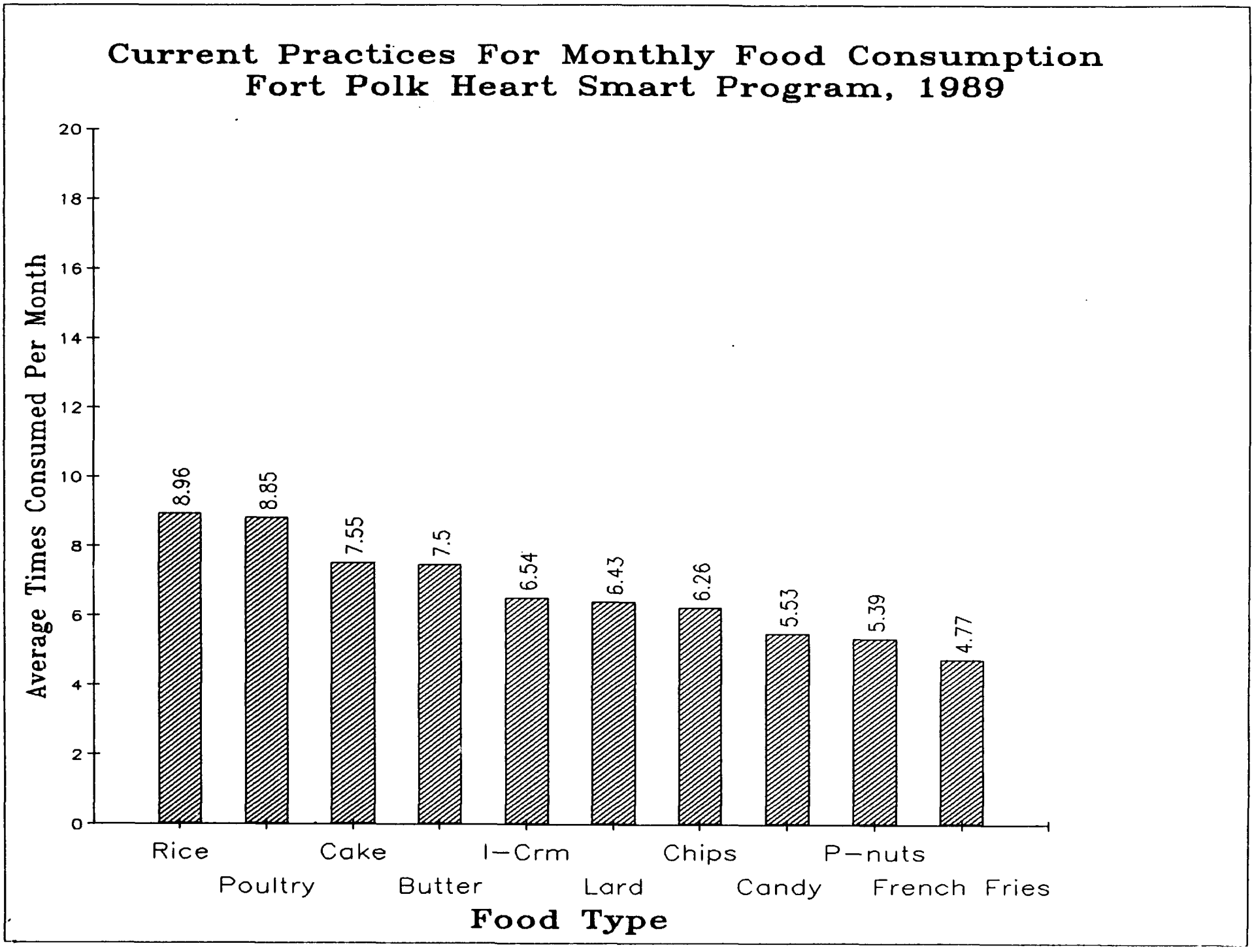


FORT POLK HEART SMART PROGRAM

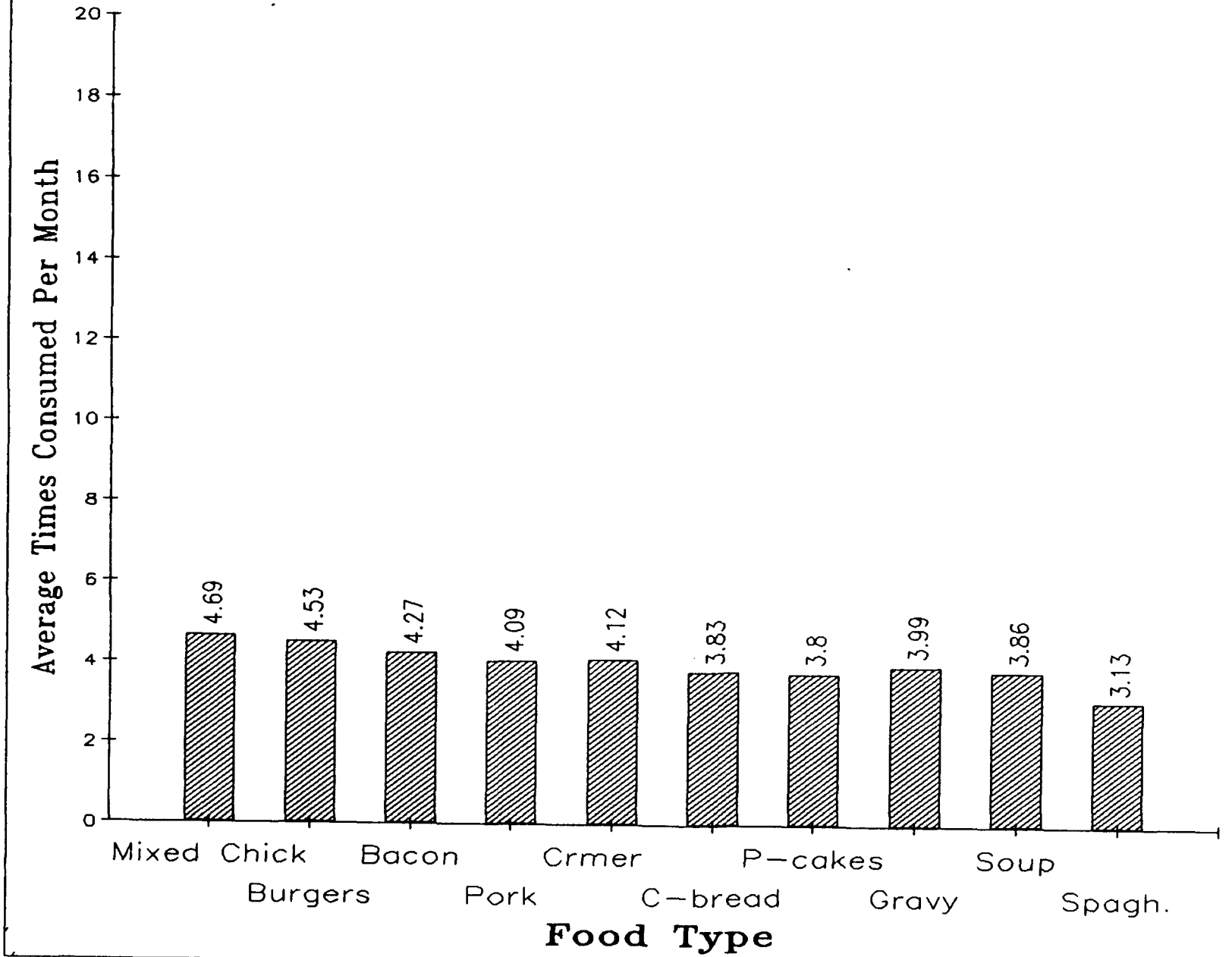
Frequency Scoring

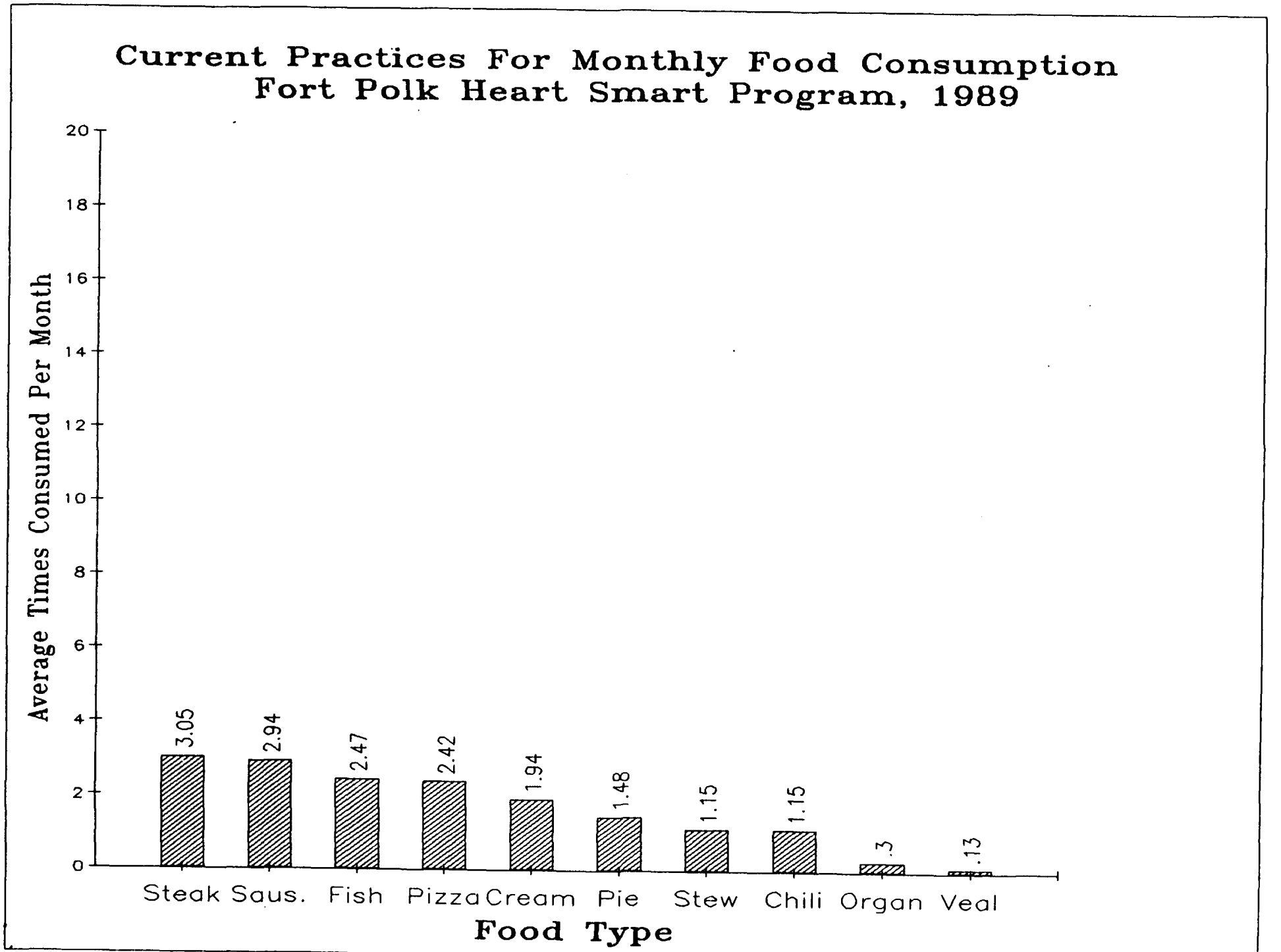
Frequency	Score
Never	0
Less than 1 time per month	.5
About 1 time per month	1
About 2-3 times per month	2.5
About 1 time per week	4
Several times per week	14
One time a day	30
More than 1 time per day	45

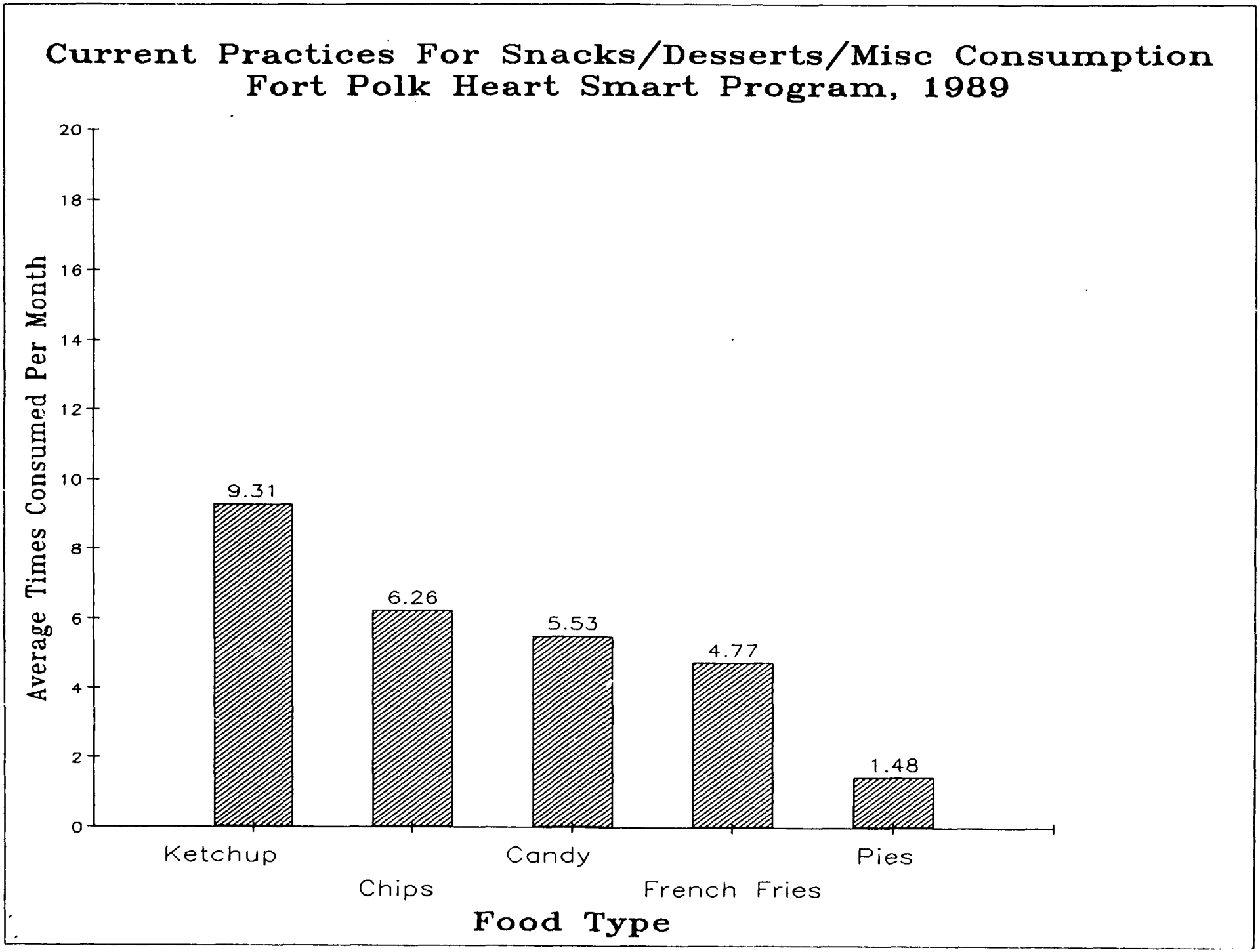

Current Practices For Monthly Food Consumption
Fort Polk Heart Smart Program, 1989
Average Times Consumed Per Month
30
25
20
15
10
5
0
26.9
20.9
20.8
17.7
12.23
11.2
10.41
10.39
10.09
9.31
Milk
Bread
Marg.
Cheese
Mayo
Cereal
Eggs
Oil
Hot Dogs
Ketchup
Food Type


Current Practices For Monthly Food Consumption
Fort Polk Heart Smart Program, 1989
Average Times Consumed Per Month
0
2
4
6
8
10
12
14
16
18
20
8.96
8.85
7.55
7.5
6.54
6.43
6.26
5.53
5.39
4.77
Rice
Poultry
Cake
Butter
I-Crm
Lard
Chips
Candy
P-nuts
French Fries
Food Type

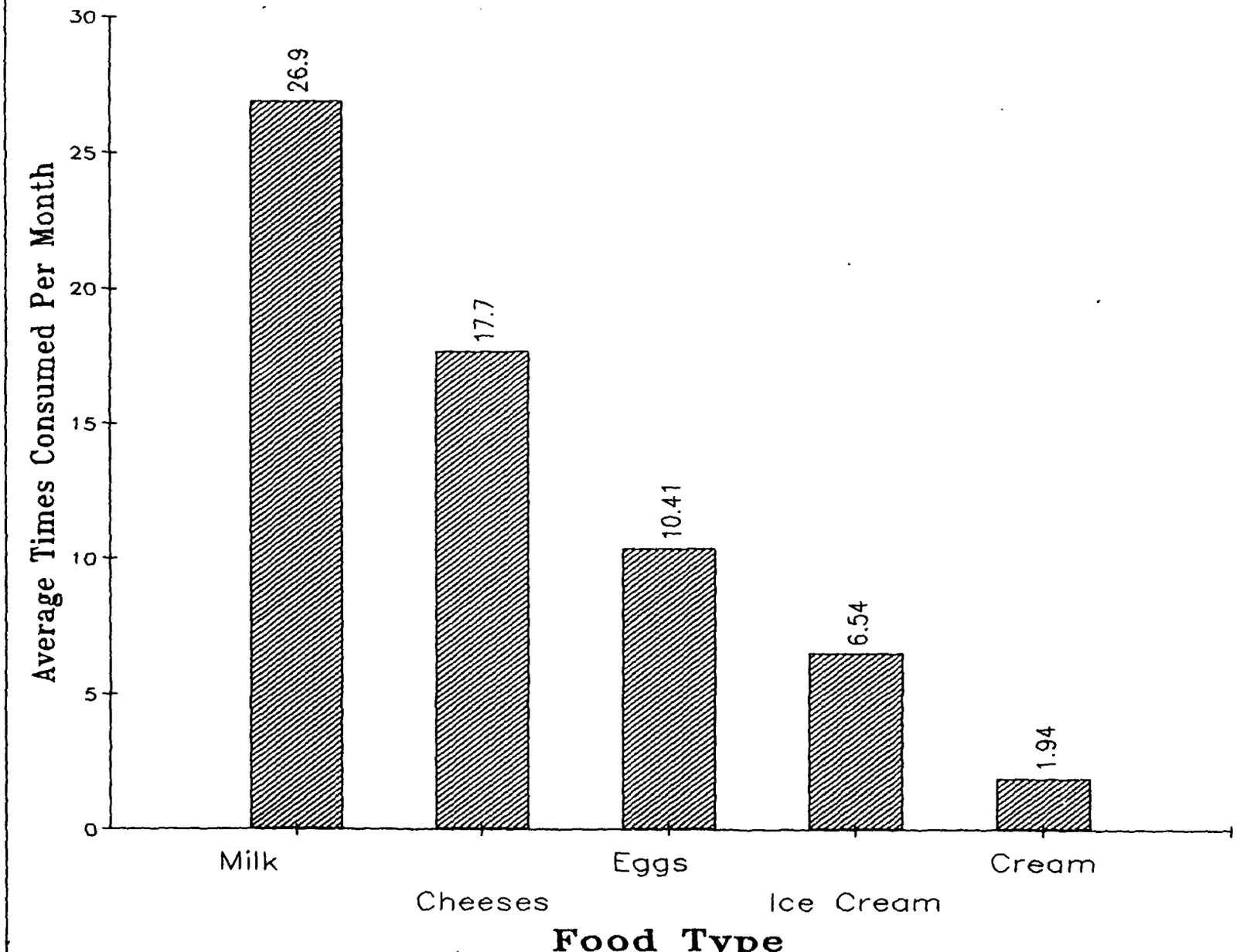
Current Practices For Monthly Food Consumption
Fort Polk Heart Smart Program, 1989

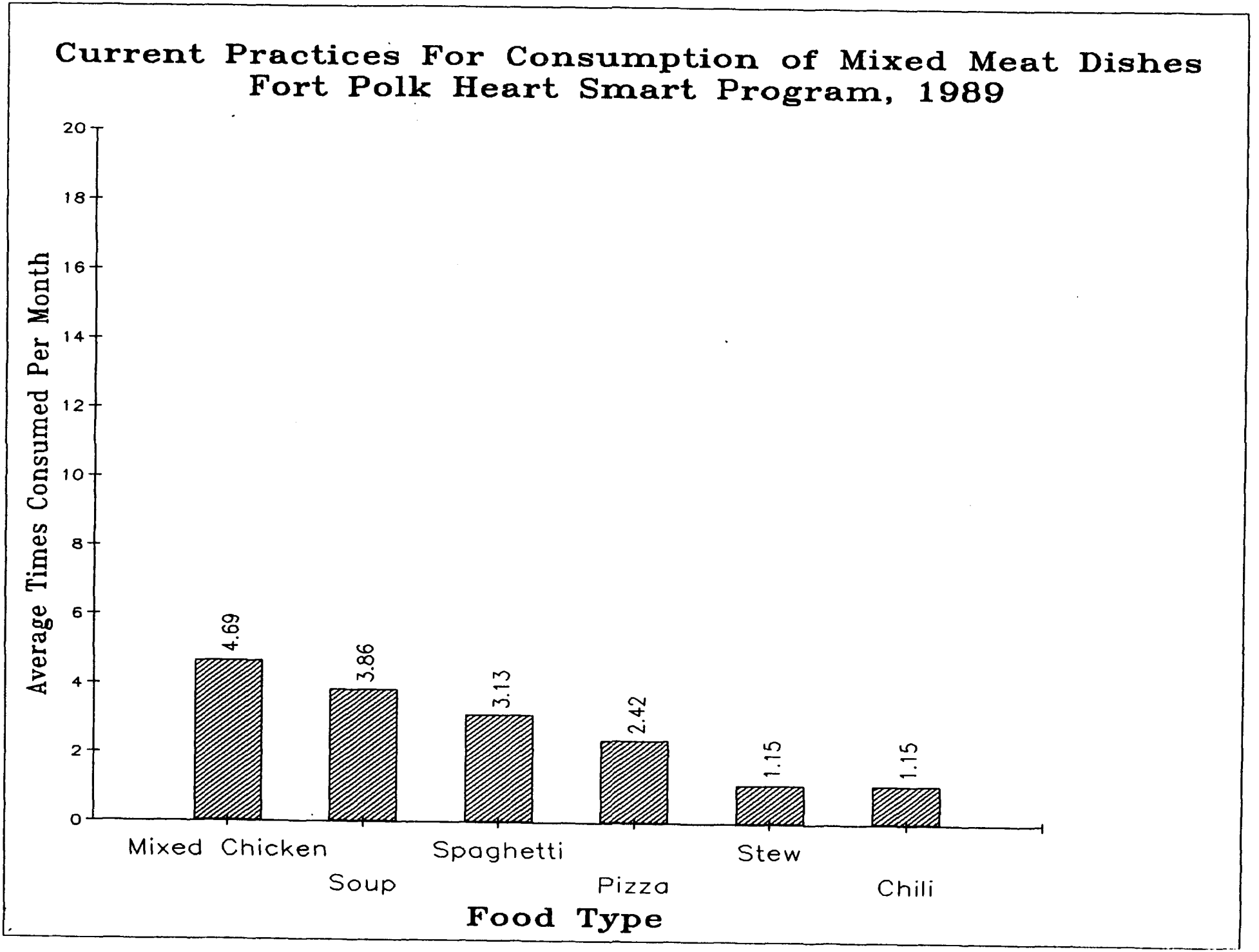


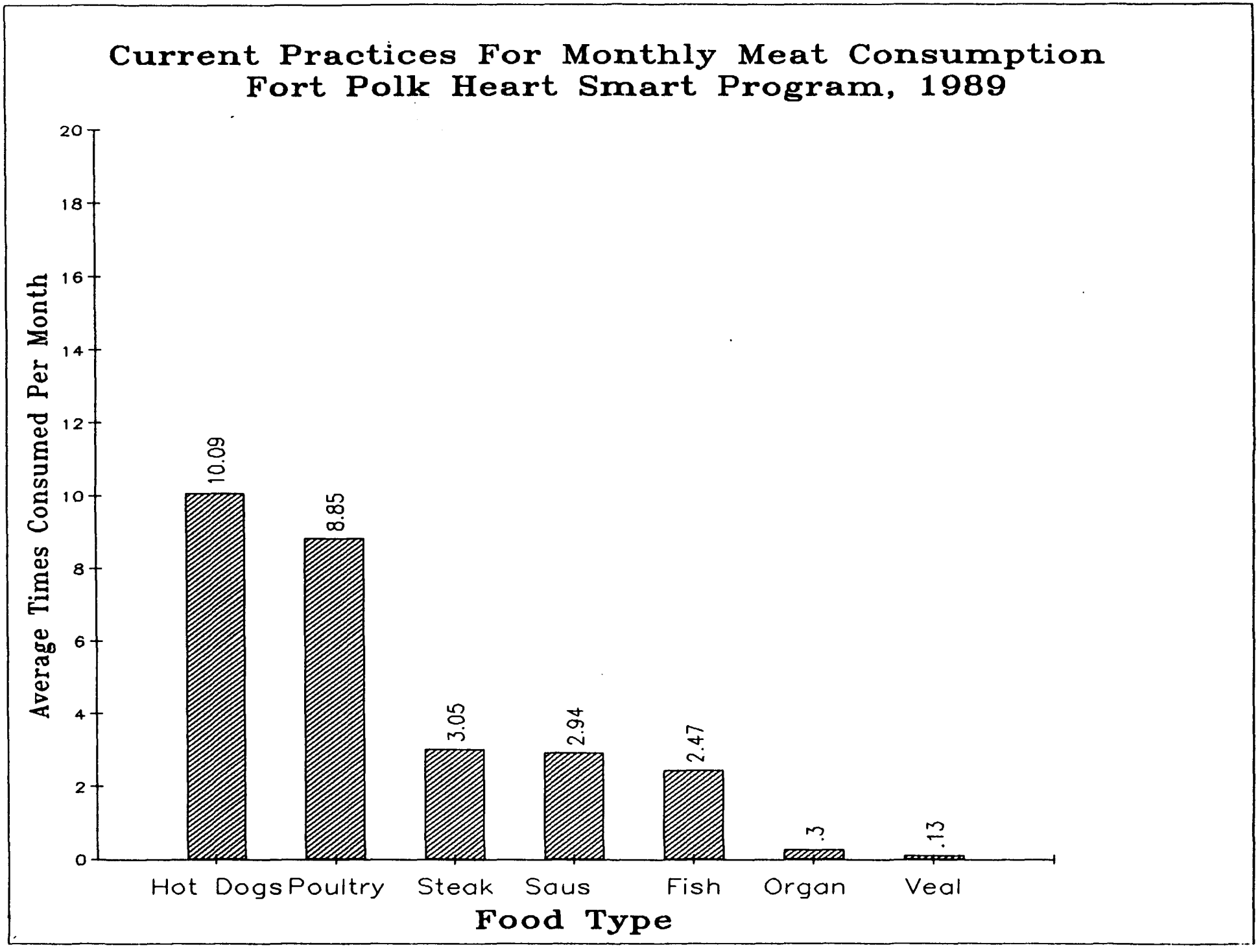

Current Practices For Monthly Food Consumption
Fort Polk Heart Smart Program, 1989
Average Times Consumed Per Month
20
18
16
14
12
10
8
6
4
2
0
3.05
2.94
2.47
2.42
1.94
1.48
1.15
1.15
.3
.13
Steak
Saus.
Fish
Pizza
Cream Pie
Stew
Chili
Organ
Veal
Food Type

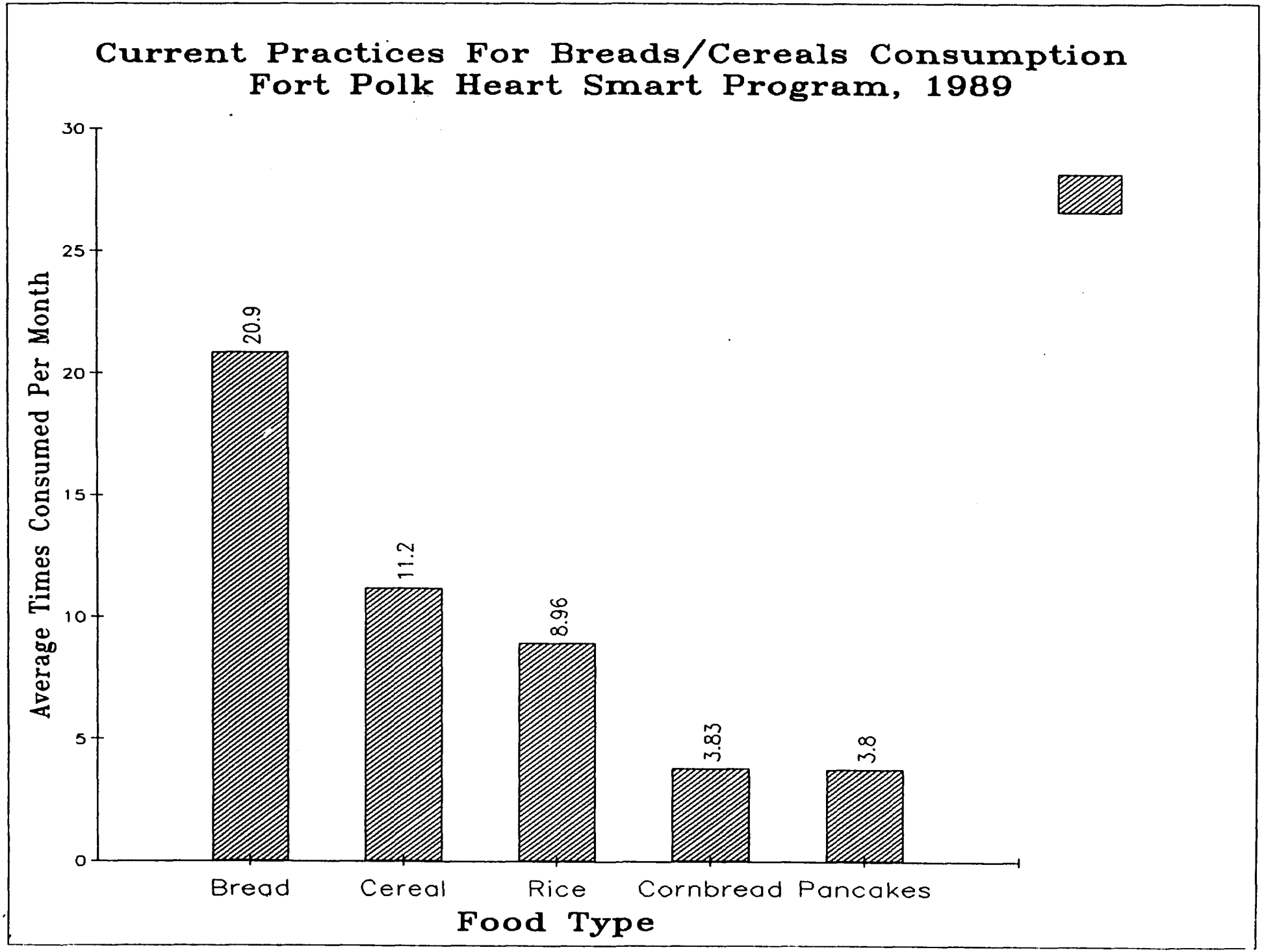

Current Practices For Snacks/Desserts/Misc Consumption
Fort Polk Heart Smart Program, 1989
Average Times Consumed Per Month
20
18
16
14
12
10
8
6
4
2
0
9.31
6.26
5.53
4.77
1.48
Ketchup
Chips
Candy
French Fries
Pies
Food Type

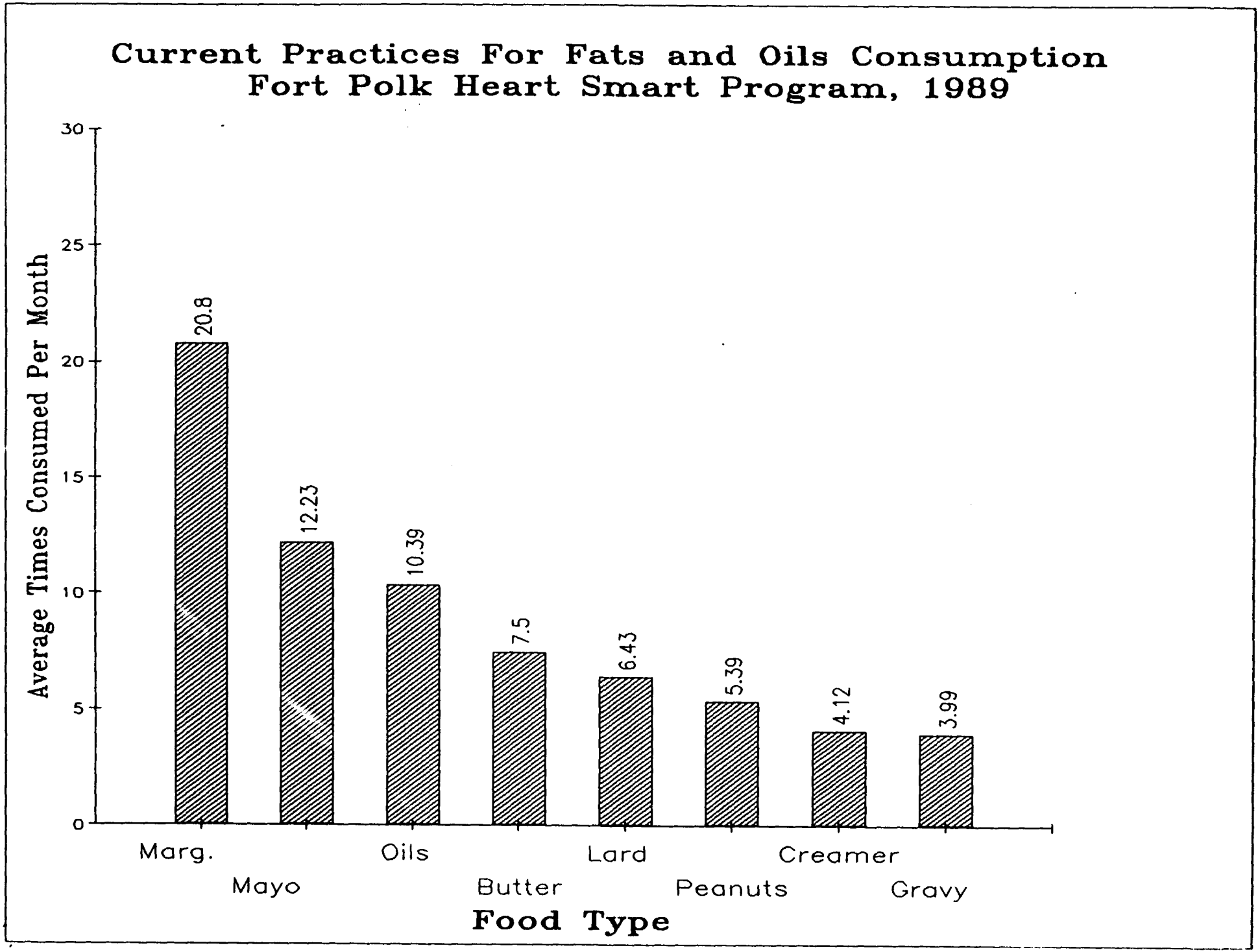
Current Practices For Consumption of Dairy Products
Fort Polk Heart Smart Program, 1989



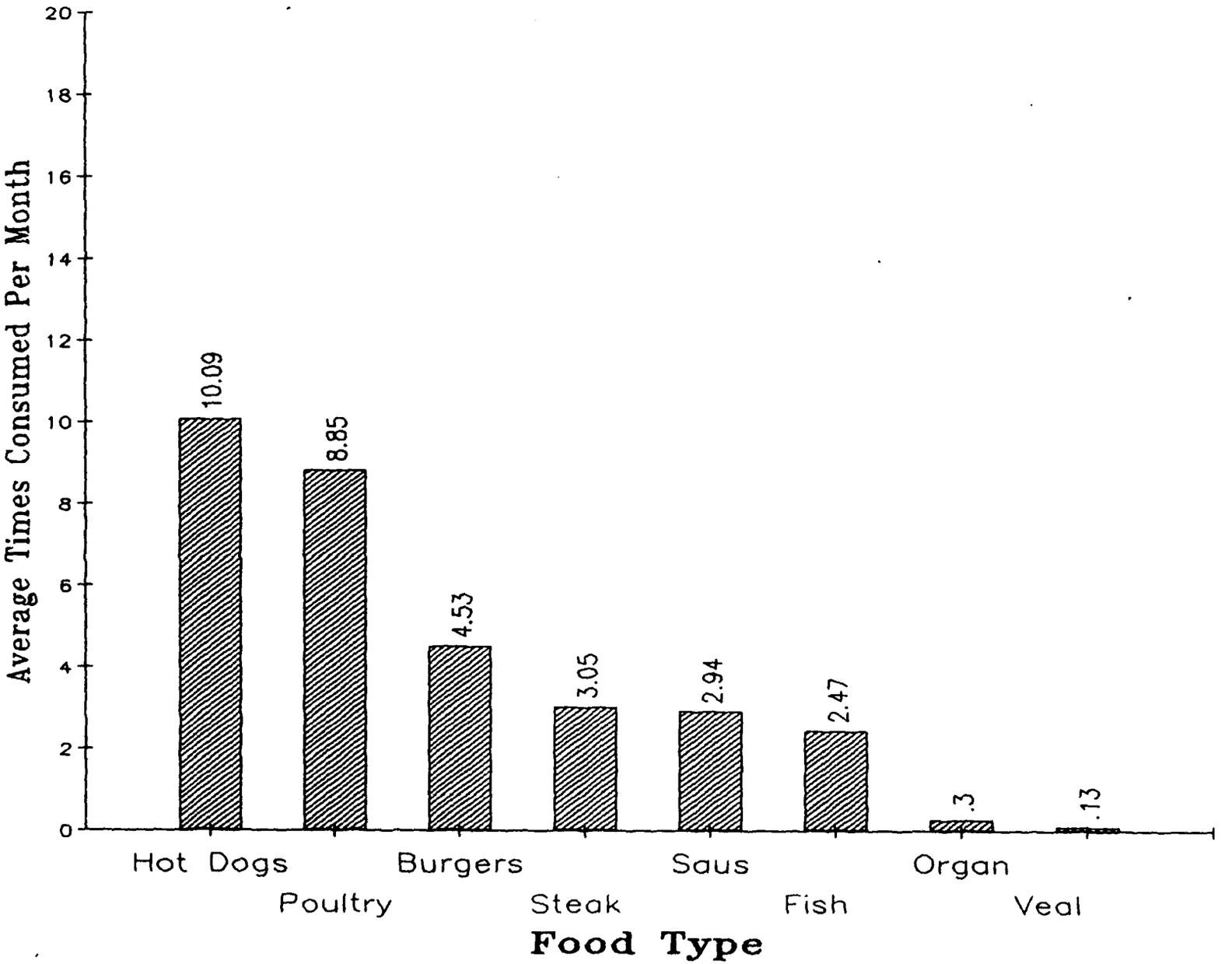

Current Practices For Consumption of Mixed Meat Dishes
Fort Polk Heart Smart Program, 1989
Average Times Consumed Per Month
20
18
16
14
12
10
8
6
4
2
0
4.69
3.86
3.13
2.42
1.15
1.15
Mixed Chicken Soup
Spaghetti
Pizza
Stew
Chili
Food Type


Current Practices For Monthly Meat Consumption
Fort Polk Heart Smart Program, 1989
Average Times Consumed Per Month
20
18
16
14
12
10
8
6
4
2
0
10.09
8.85
3.05
2.94
2.47
.3
.13
Hot Dogs
Poultry
Steak
Saus
Fish
Organ
Veal
Food Type

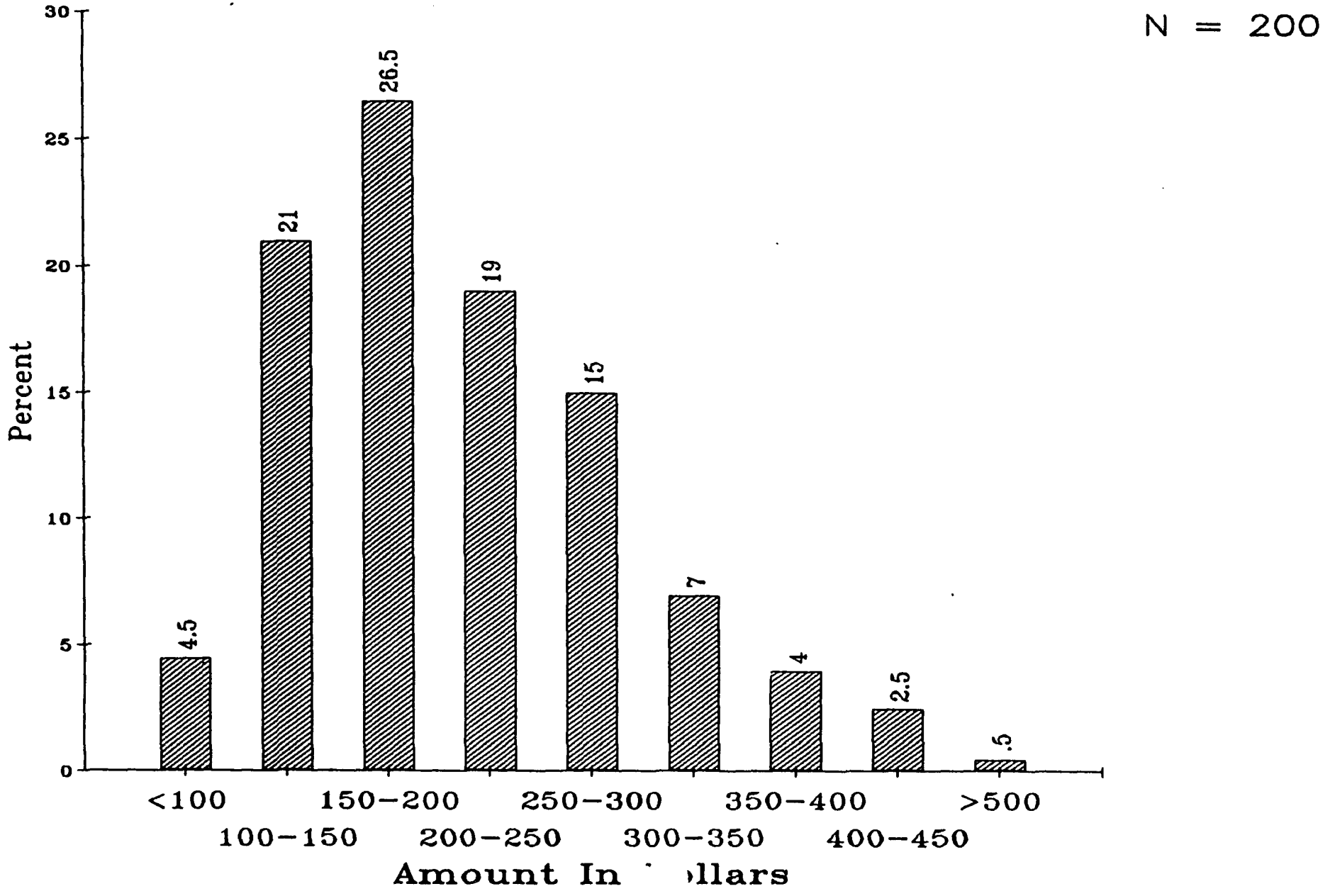

Current Practices For Breads/Cereals Consumption
Fort Polk Heart Smart Program, 1989
Average Times Consumed Per Month
30
25
20
15
10
5
0
20.9
11.2
8.96
3.83
3.8
Bread
Cereal
Rice
Cornbread
Pancakes
Food Type


Current Practices For Fats and Oils Consumption
Fort Polk Heart Smart Program, 1989
Average Times Consumed Per Month
30
25
20
15
10
5
0
20.8
12.23
10.39
7.5
6.43
5.39
4.12
3.99
Marg.
Mayo
Oils
Butter
Lard
Peanuts
Creamer
Gravy
Food Type

Current Practices For Monthly Meat Consumption
Fort Polk Heart Smart Program, 1989

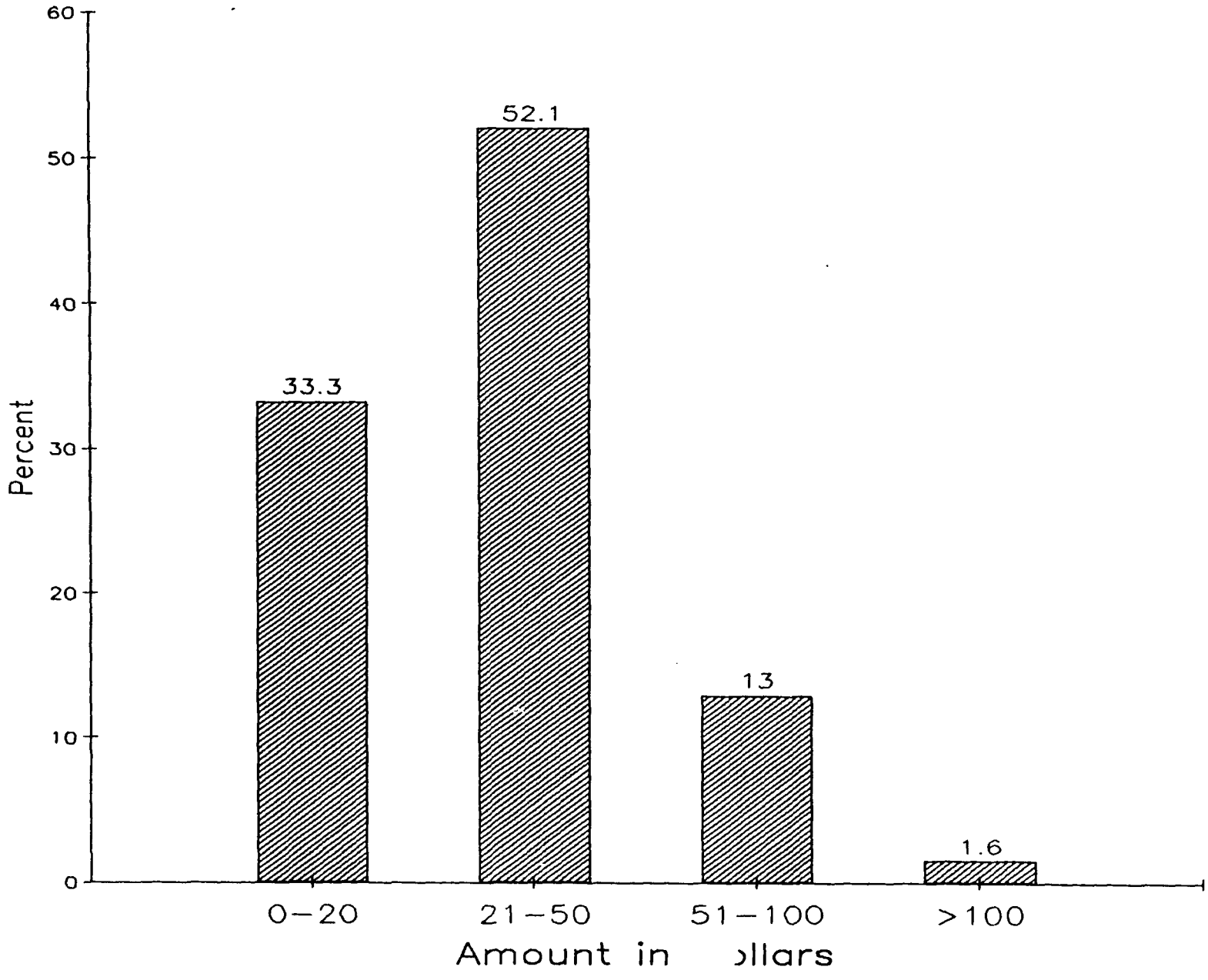


Grocery Expenditures of Military Wives
Fort Polk Heart Smart Program, Project 1, 1989

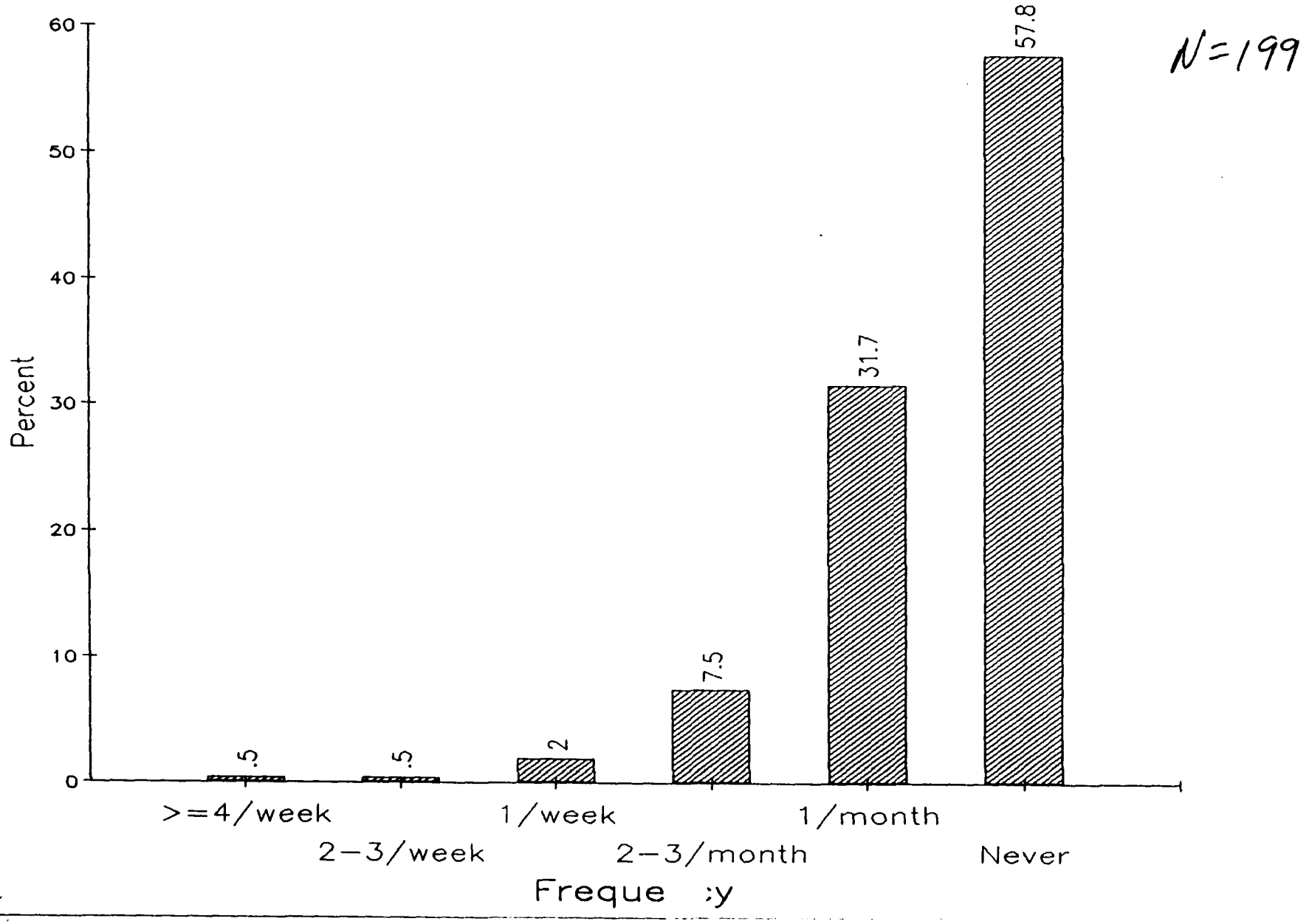


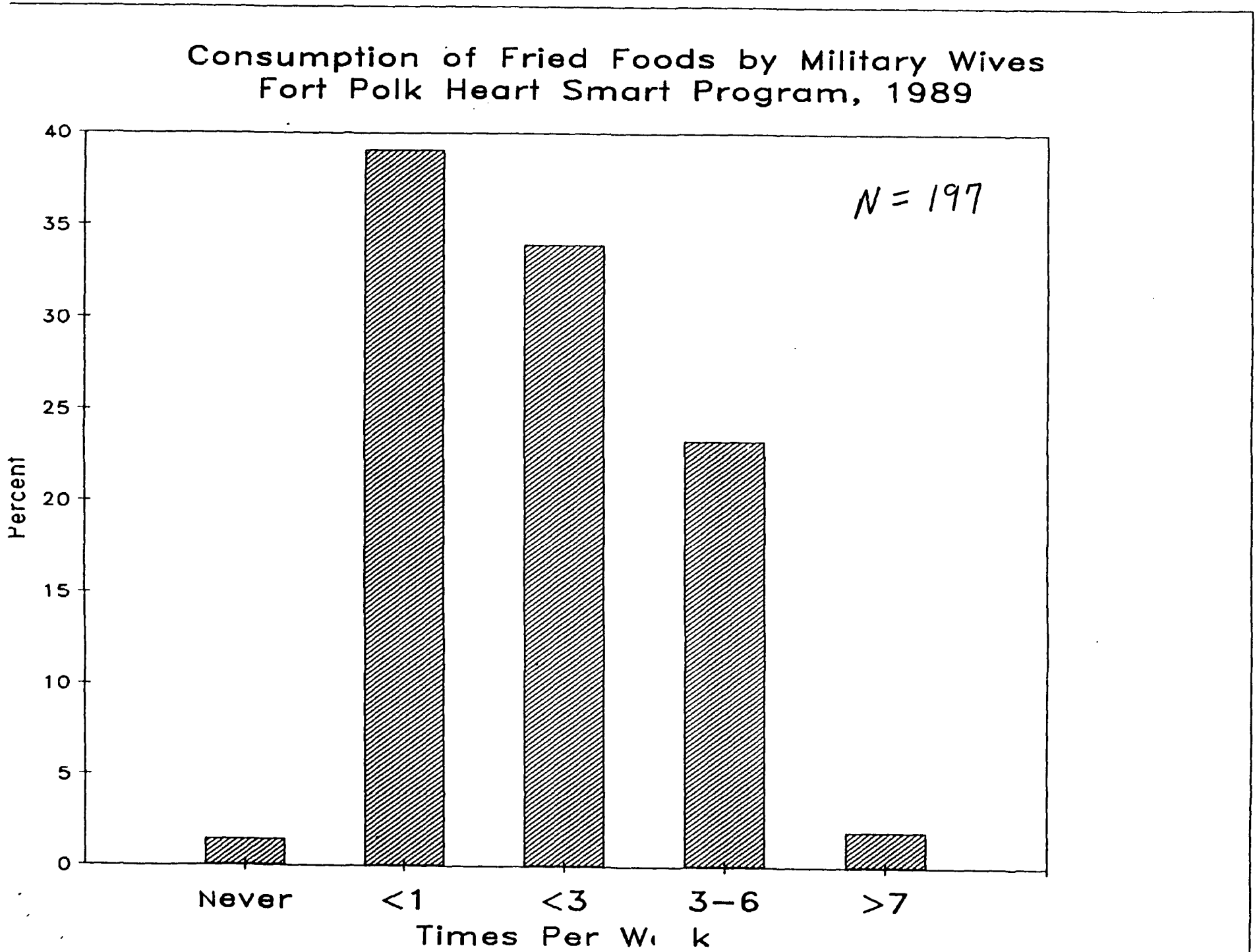
Monthly Expenses for Restaurant Dining
Fort Polk Heart Smart Program, 1989

N = 192



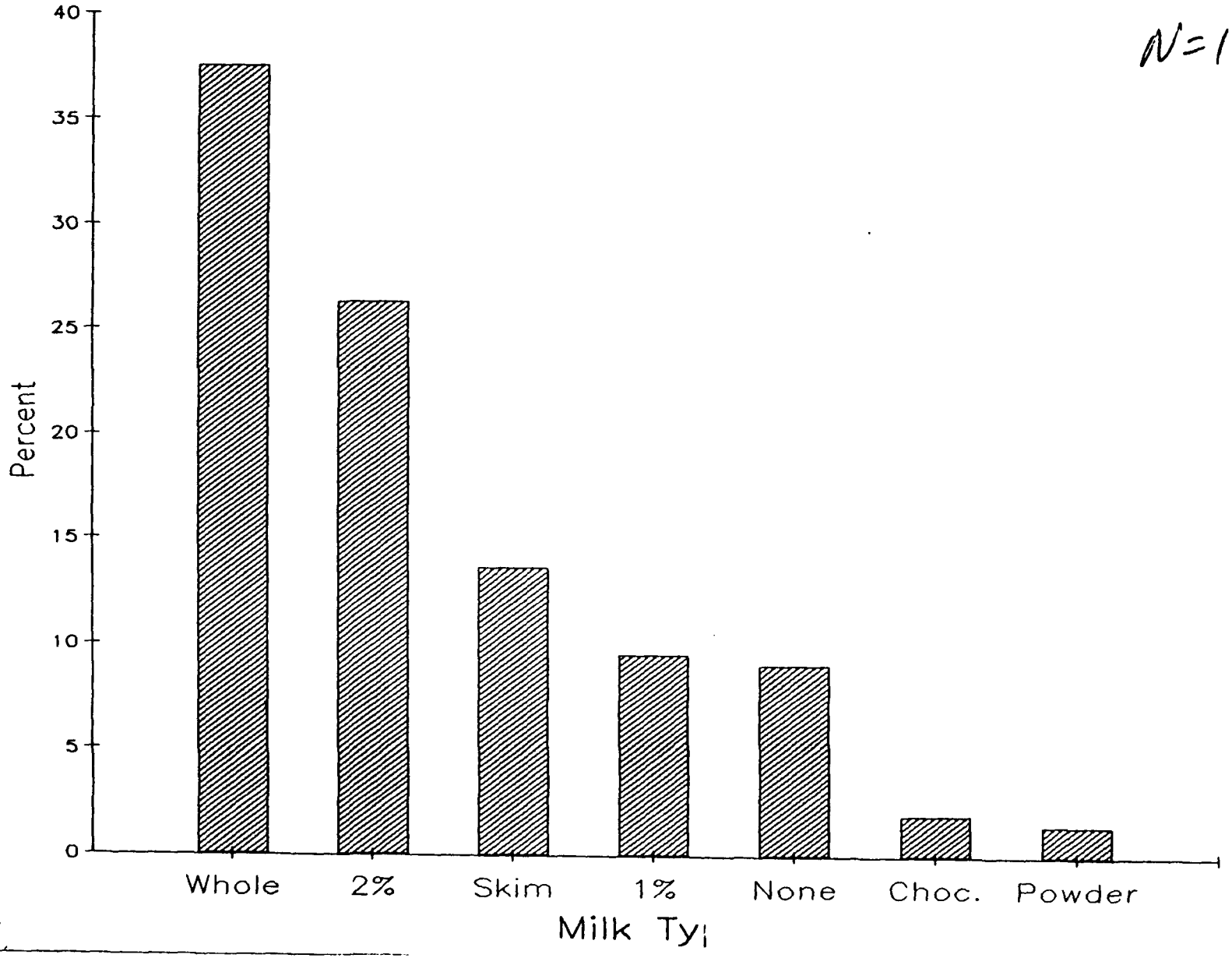
TV Dinner Consumption by Spouses
Fort Polk Heart Smart Project, 1989



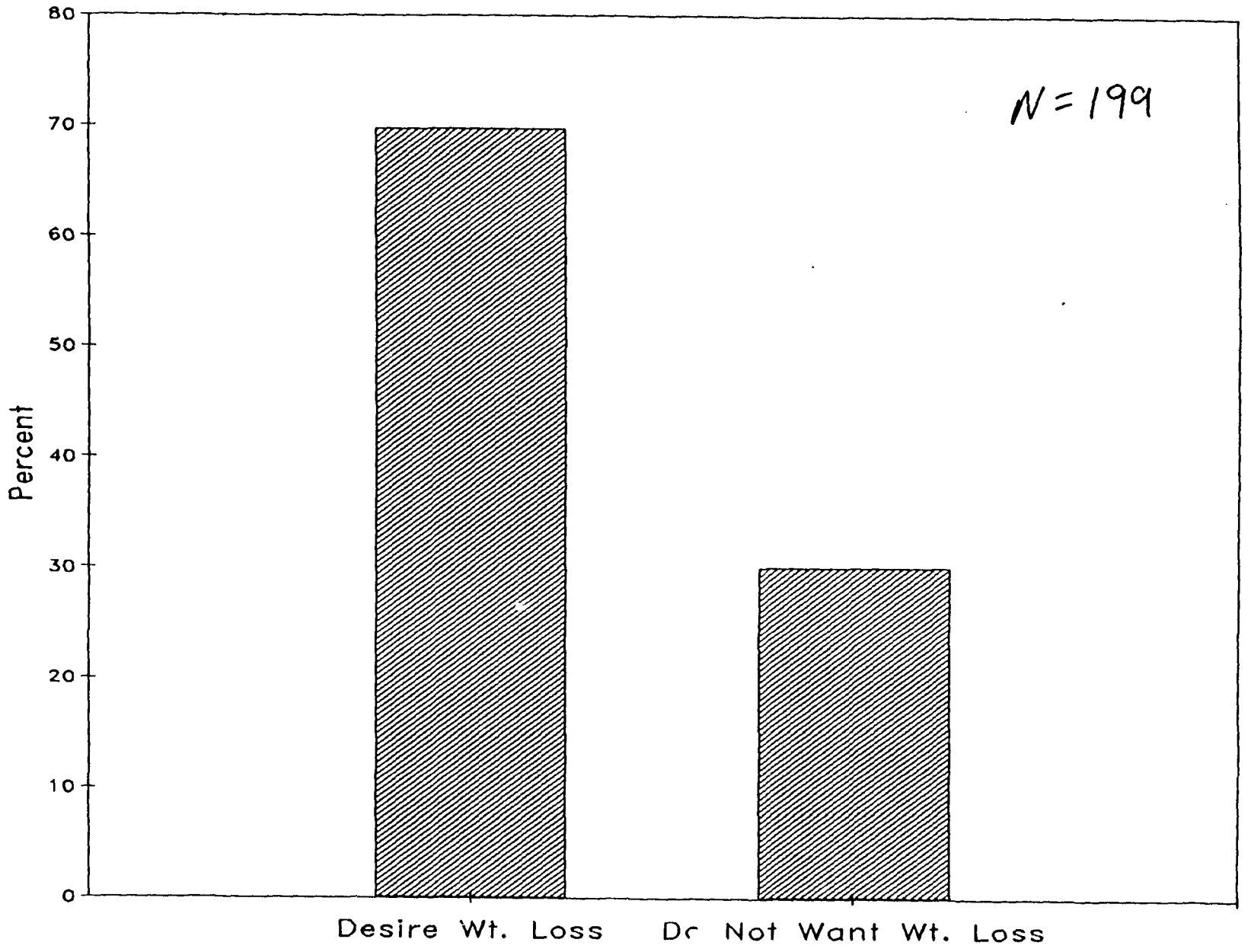

Consumption of Fried Foods by Military Wives
Fort Polk Heart Smart Program, 1989
N = 197
Percent
40
35
30
25
20
15
10
5
0
Never
<1
<3
3–6
>7
Times Per Week

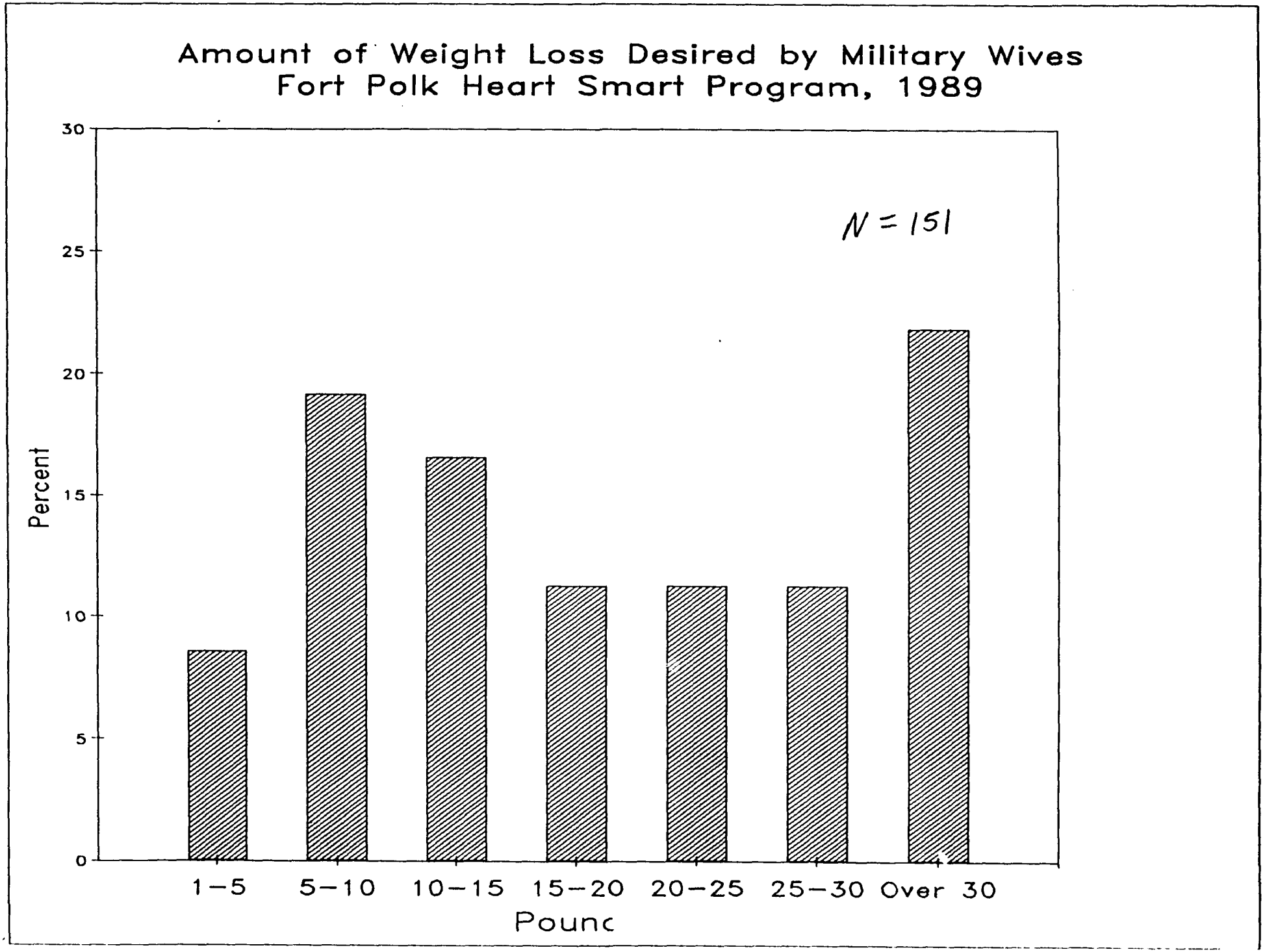
Milk Type Usually Consumed By Spouses Fort Polk Heart Smart Project, 1989

N=197



Percentage of Military Wives Who Desire Weight loss
Fort Polk Heart Smart Program, 1989




Amount of Weight Loss Desired by Military Wives
Fort Polk Heart Smart Program, 1989
N = 151
Percent
30
25
20
15
10
5
0
1–5
5–10
10–15
15–20
20–25
25–30
Over 30
Pounc

Nutrient Intake by Age

Dietary Component	Age Group 19-24 (n=51)	Age Group 25-45 (n=135)
Calories, kcal	2015	1812
Protein	71.3	63.5
Animal	50.1	41.6
Vegetable	18.5	17.7
Fat	78.6	72.0
SFA	27.0	26.0
PUFA	14.6	14.6
MONO	27.9	24.3
Carbohydrate	269.7	233.3
Starch	150.7	86.4
Sugar	128.2	114.5
Sucrose	79.4	71.3
Fiber	3.14	2.84
Cholesterol	229	243

Percent Contribution of Macronutrients to Energy Intake by Age

	Age Group	
Dietary Component	19-24	25-45
Calories	2015	1812
% Protein	14.8	14.2
% Fat	34.2	34.9
% SFA	11.9	12.9
% CHO	53.9	52.4
% Alcohol	0.14	0.47

Electrolyte and Iron Intake by Age

Dietary Component	Age Group 19-24	25-45
Sodium	3418	3111
Potassium	2352	2170
Magnesium	222	222
Calcium	933	803
Phosphorus	1250	1070
Iron	.016	.022

Nutrient Intake by Ethnic Origin

Dietary Component	Ethnicity				
	White (n=125)	Black (n=34)	Hispanic (n=20)	Asian (n=3)	Other (n=4)
Calories, kcal	1906	1771	1754	2018	1946
Protein	66.1	59.6	63.7	104.6	81.3
Animal	43.1	39.7	47.8	77.2	64.3
Vegetable	18.3	17.5	15.0	26.2	15.9
Fat	75.2	68.1	70.4	68.2	101.2
SFA	26.9	24.1	24.2	29.4	32.8
PUFA	15.2	13.0	14.3	5.6	18.6
MONO	25.4	24.3	23.6	24.8	40.0
Carbohydrate	249.4	239.4	219.6	251.3	196.3
Starch	103.9	132.7	63.0	111.4	65.1
Sugar	122.8	113.9	109.8	98.8	69.9
Sucrose	75.5	78.9	60.8	50.1	46.0
Fiber	3.06	2.35	2.27	7.44	3.23
Cholesterol	216	235	301	610	431

Percent Contribution of Macronutrients to Energy Intake by Ethnic Origin

	Ethnicity				
Dietary Component	White	Black	Hispanic	Asian	Other
Calories, kcal	1906	1771	1754	2018	1946
% Protein	14.2	13.4	15.0	20.5	16.5
% Fat	34.6	33.6	36.3	30.2	43.1
% SFA	12.9	11.4	12.6	13.0	13.6
% CHO	53.0	55.1	49.2	50.3	45.1
% Alcohol	0.43	0.19	0.51	0.0	0.0

Electrolyte and Iron Intake by Ethnic Origin

Dietary Component	Ethnicity				
	White	Black	Hispanic	Asian	Other
Sodium	3303	2863	2498	6865	3390
Potassium	2375	1728	1724	4103	2597
Magnesium	238	181	162	415	235
Calcium	914	667	686	1123	506
Phosphorus	1172	979	990	1445	1095
Iron	.024	.014	.010	.022	.011

Age Range and Median of Military Dependents by Husband's Rank

Husband's Rank	(N)	Age Range (yrs)	Median Age (yrs)
Enlisted	(49)	19-46	23
Enlisted (Sergeant & Higher)	(107)	20-42	28.5
Officers	(29)	23-45	34

Nutrient Intake by Husband's Rank

Dietary Component	Husband's Rank		
	Enlisted (n=49)	Enlisted (Sergeant & Higher) (n=107)	Officers (n=29)
Calories, kcal	2101	1768	1870
Protein	69.2	64.5	64.4
Animal	48.5	42.5	42.2
Vegetable	17.9	18.3	16.6
Fat	85.2	68.3	75.4
SFA	29.5	23.3	32.1
PUFA	17.1	13.3	15.2
MONO	29.7	23.6	24.1
Carbohydrate	277.5	230.0	239.6
Starch	118.4	108.1	67.0
Sugar	139.9	107.6	123.6
Sucrose	96.9	65.4	65.8
Fiber	2.74	2.91	3.32
Cholesterol	253	239	199

Percent Contribution of Macronutrients to Energy Intake by Husband's Rank

Dietary Component	Husband's Rank		
	Enlisted	Enlisted (Sergeant & Higher)	Officers
Calories	2101	1768	1870
% Protein	13.6	14.8	13.8
% Fat	35.7	34.2	34.3
% SFA	12.7	11.5	16.3
% CHO	52.8	52.8	53.4
% Alcohol	0.47	0.37	0.29

Electrolyte and Iron Intake by Husband's Rank

Dietary Component	Husband's Rank		
	Enlisted	Enlisted (Sergeant & Higher)	Officers
Sodium	3531	3044	3195
Potassium	2167	2227	2303
Magnesium	218	222	232
Calcium	899	797	913
Phosphorus	1189	1095	1104
Iron	.019	.023	.014

Nutrient Intake by Meal Period

Dietary Component	Meal Period			
	Breakfast (n=157)	Lunch (n=162)	Dinner (n=184)	Snacks (n=170)
Calories, kcal	346	529	700	462
Protein	12.3	19.9	30.4	8.6
Animal	7.5	12.8	22.8	4.3
Vegetable	3.7	5.1	6.9	3.9
Fat	12.2	21.5	31.4	15.1
SFA	5.6	7.4	10.8	4.9
PUFA	2.1	4.2	6.1	3.5
MONO	4.0	7.3	11.1	5.0
Carbohydrate	48.0	66.4	75.8	76.5
Starch	32.9	28.1	30.9	23.2
Sugar	22.3	29.9	29.2	48.7
Sucrose	10.8	20.0	18.9	30.9
Fiber	0.39	0.79	1.22	0.76
Cholesterol, mg	72	66	99	26

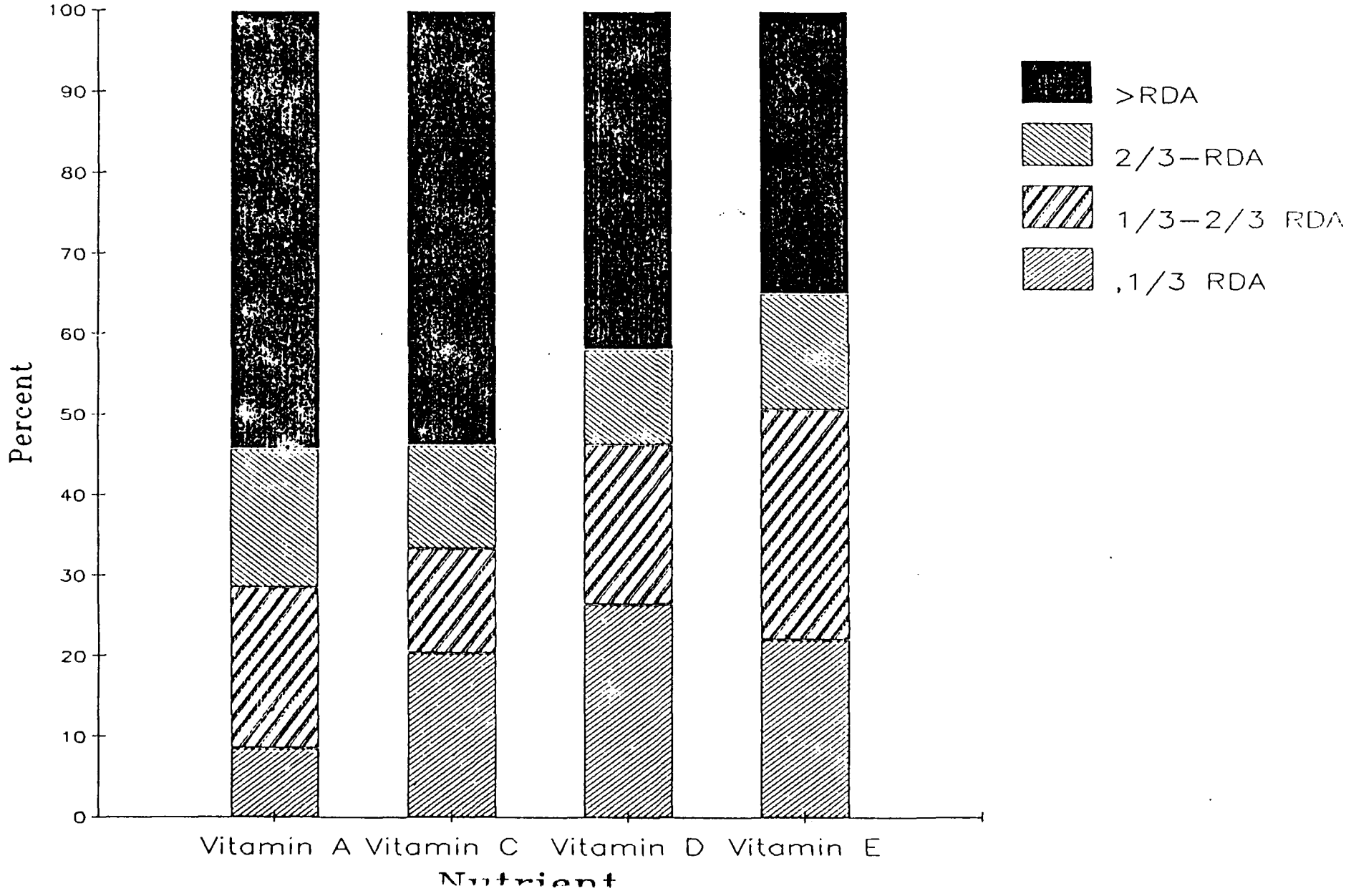
Percent Contribution of Macronutrients to Energy Intake by Meal Period

	Meal Period			
Dietary Component	Breakfast	Lunch	Dinner	Snacks
Calories	346	529	700	462
% Protein	14.1	15.2	17.6	7.6
% Fat	28.6	34.9	37.2	26.1
% SFA	22.1	11.8	12.4	9.1
% CHO	58.8	52.0	46.0	69.7
% Alcohol	0.29	0.01	0.49	0.89

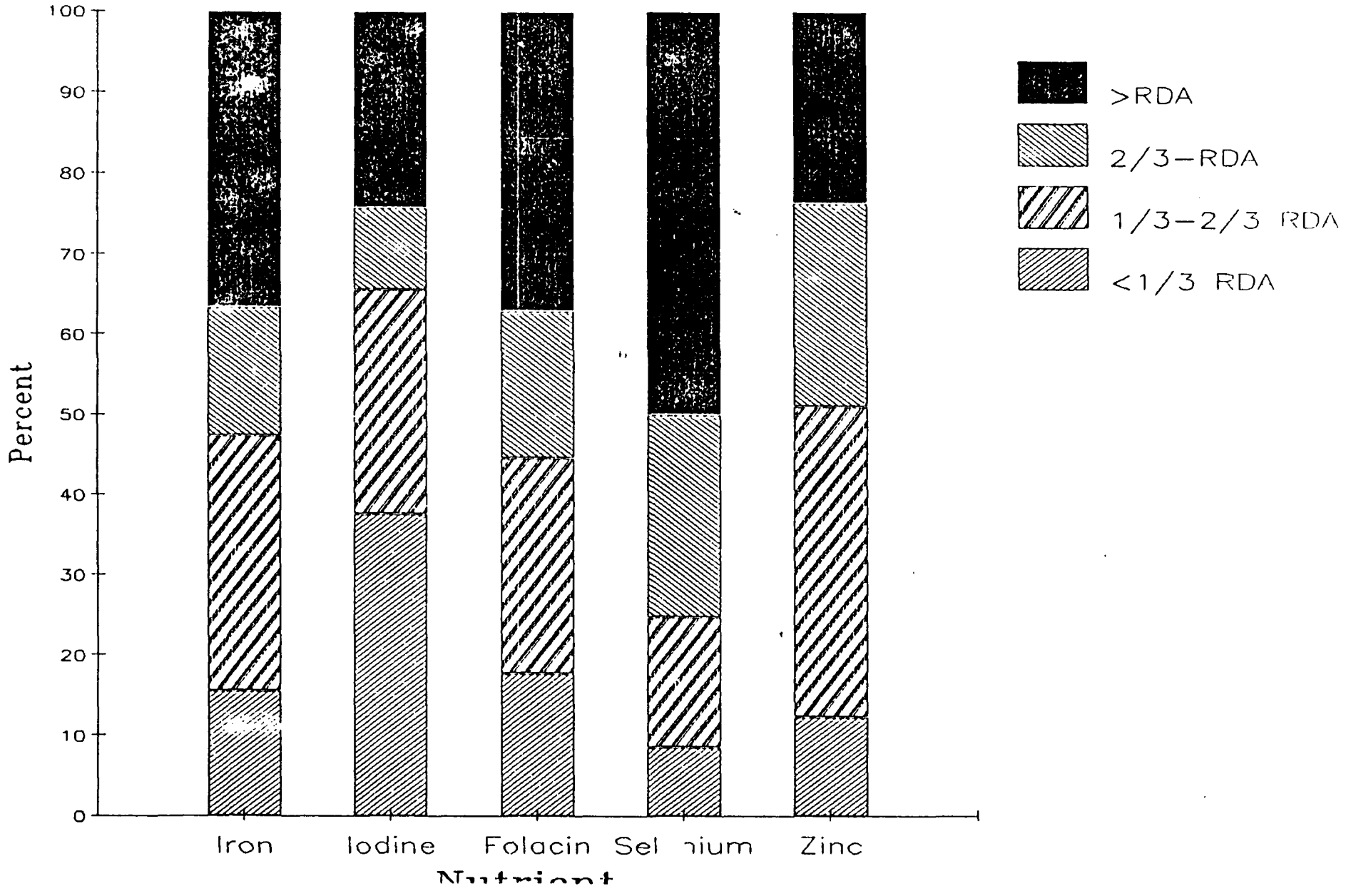
Electrolyte and Iron Intake by Meal Period

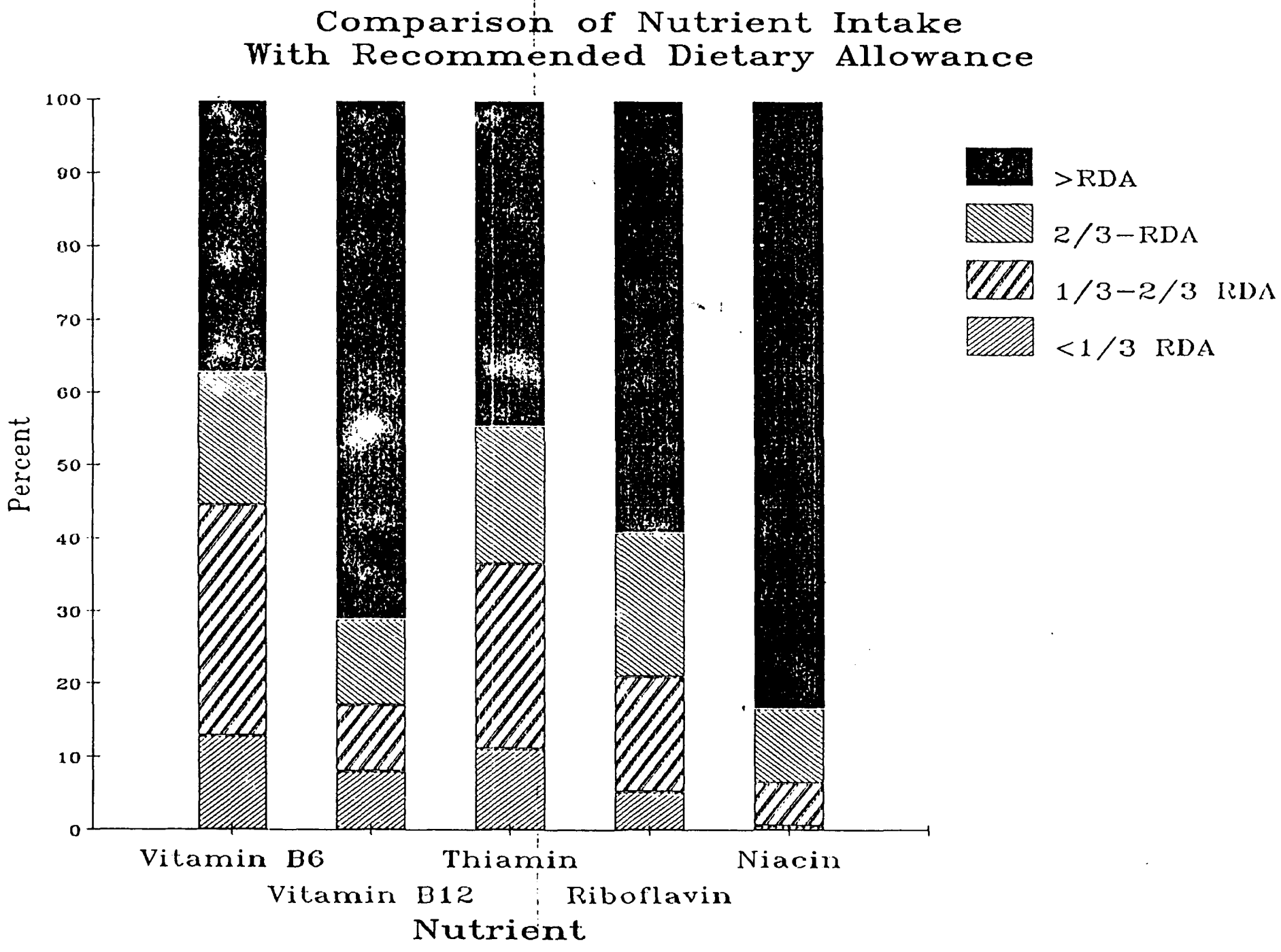
Dietary Component	Meal Period			
	Breakfast	Lunch	Dinner	Snacks
Sodium	506	1161	1432	373
Potassium	533	542	857	492
Magnesium	54	56	79	53
Calcium	261	215	258	192
Phosphorus	273	308	427	218
Iron	.008	.003	.004	.007

Comparison of Nutrient Intake With Recommended Dietary Allowance

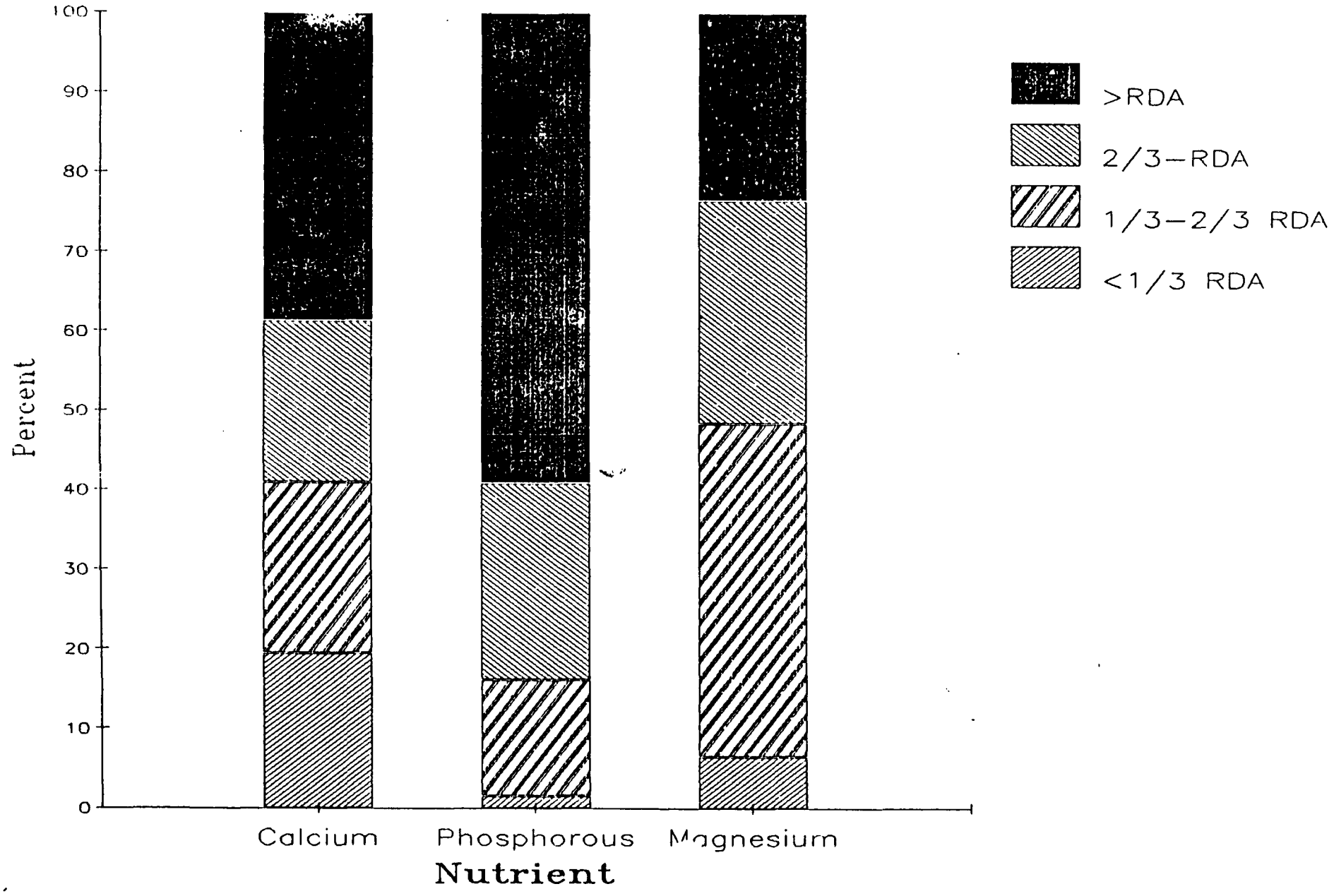


Comparison of Nutrient Intake With Recommended Dietary Allowance




Comparison of Nutrient Intake
With Recommended Dietary Allowance
Percent
100
90
80
70
60
50
40
30
20
10
0
>RDA
2/3-RDA
1/3-2/3 RDA
<1/3 RDA
Vitamin B6
Thiamin
Niacin
Vitamin B12
Riboflavin
Nutrient

Comparison of Nutrient Intake With Recommended Dietary Allowance



MEAN DIETARY INTAKES OF DEPENDENTS OF MILITARY PERSONNEL COMPARED WITH THREE NATIONAL SURVEYS

(n=188)

Dietary Component (g)	Fort Polk 1989	NHANES II 1976-1980	LRC 1982	CSFII 1985	CSFII 1986
Calories, kcal	1869	1643	1984	1528	1473
Protein	66	64	75	61	60
Fat	74	68	90	63	61
SFA	26	24	34	23	22
PUFA	15		16	13	12
MUFA	25		34	23	22
Carbohydrate	243	187	205	177	169
Starch	104				
Total Sugar	118				
Sucrose	73				
Fiber	2.9				
Cholesterol, mg	239	278	332	280	272

NHANES II - Second National Health and Nutrition Examination Survey - Women 25-34 years.

LRC - Lipid Research Clinics - White women - 25-29 yrs.

CSFII - Women 19-50 yrs. - Nationwide Food Consumption Survey - Continuing Survey of Food Intakes by Individuals (an average of 4 days).

MEAN DIETARY INTAKES OF DEPENDENTS OF MILITARY PERSONNEL
COMPARED WITH THREE NATIONAL SURVEYS
(n=188)

Dietary Component	Fort Polk 1989	NHANES II 1976-1980	LRC 1982	CSFII 1985	CSFII 1986
Calories, kcal	1869	1643	1984	1528	1473
Protein, % kcal	14.3	--	15.4	16.2	16.7
Carbohydrate, %kcal	52.7	--	41.9	46.4	45.9
Fat, %kcal	34.7	36.3	39.9	36.8	36.7
Alcohol, %kcal	0.4	--	--	--	--

NHANES II - Second National Health and Nutrition Examination Survey - Women 19-50 yrs.

LRC - Lipid Research Clinics - White women - 25-29 yrs.

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MEAN DIETARY INTAKES OF DEPENDENTS OF MILITARY PERSONNEL
COMPARED WITH THREE NATIONAL SURVEYS
(n=188)

	Fort Polk	NHANES II	LRC	CSFII	
Dietary Component (g)	1989	1976-1980	1982	1985	1986
Calories, kcal	1869	1643	1984	1528	1473
Protein	66	64	75	61	60
Fat	74	68	90	63	61
SFA	26	24	34	23	22
PUFA	15		16	13	12
MUFA	25		34	23	22
Carbohydrate	243	187	205	177	169
Starch	104				
Total Sugar	118				
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Fiber	2.9				
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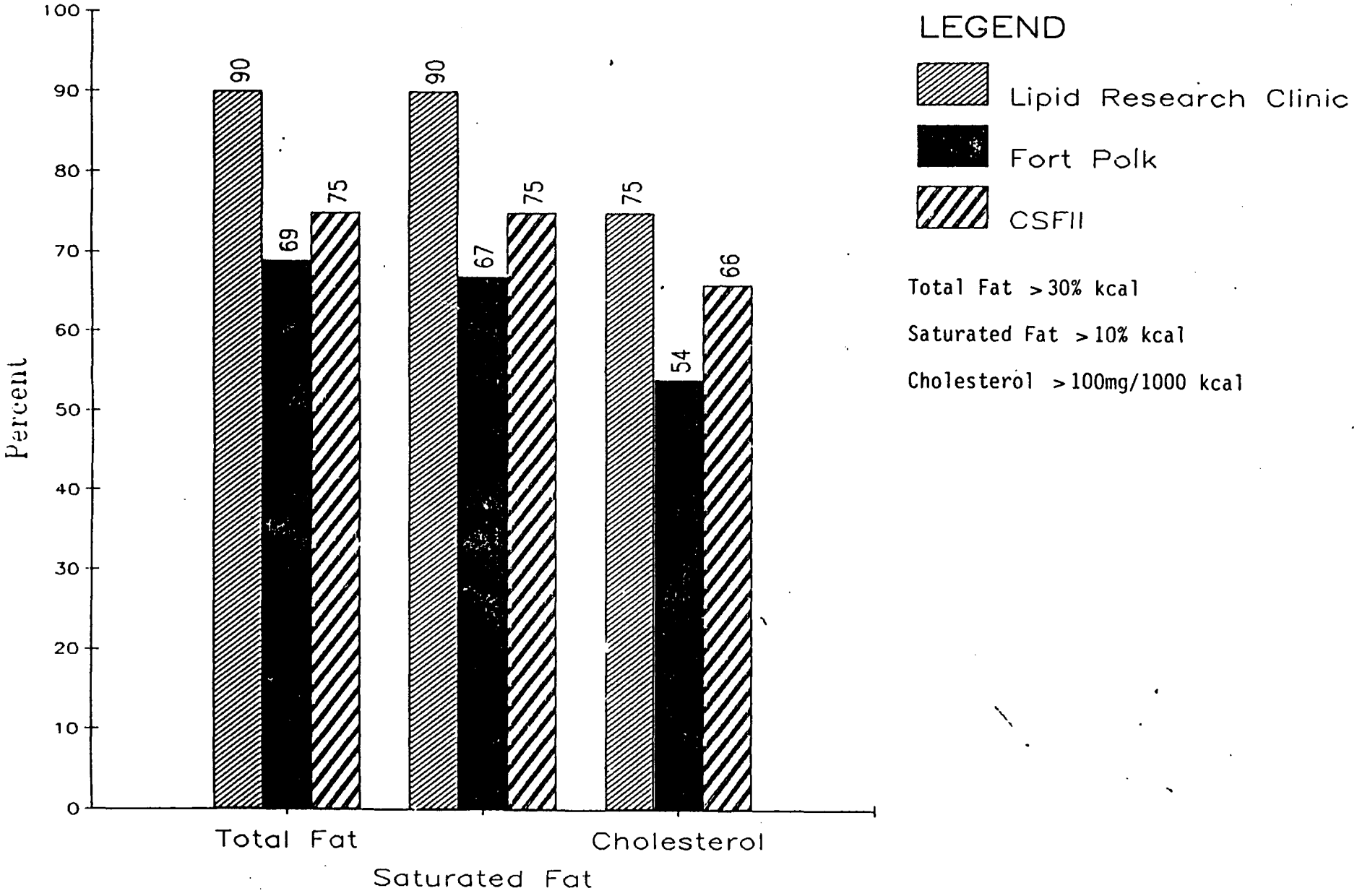
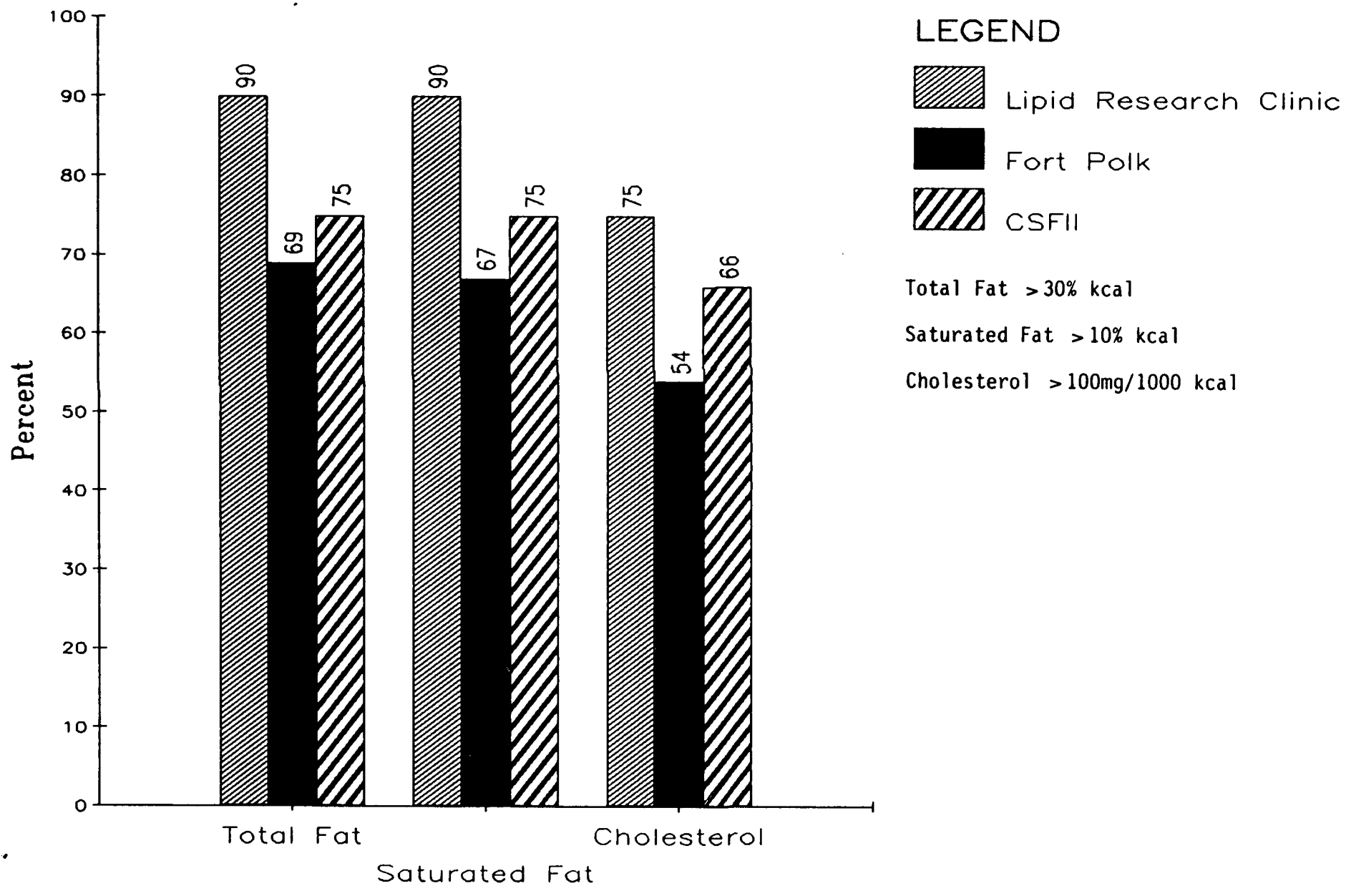
Comparison of Army Wives vs. 2 National Health Surveys
Percent Not Meeting AHA Dietary Recommendations



Comparison of Army Wives vs. 2 National Health Surveys
Percent Not Meeting AHA Dietary Recommendations



MEAN DIETARY INTAKES OF DEPENDENTS OF MILITARY PERSONNEL COMPARED WITH TWO NATIONAL SURVEYS

(n=188)

Dietary Component (mg)	Fort Polk	NHANES II	CSFII	
	1982	1976-1980	1985	1986
Sodium, g	3.2	2.4	2.4	2.3
Potassium, g	2.2	2.1	2.1	2.1
Calcium	834	636	614	621
Phosphorus	1118	1021	966	958
Magnesium	221	--	207	205
Iron	20	11	10	10

CSFII - Women 19-50 yrs. - Nationwide Food Consumption Survey - Continuing Survey of Food Intakes by Individuals (an average of 4 days).

NHANES II - Second National Health and Nutrition Examination Survey - Women 25-34 years.